Asian Journal of Food Research and Nutrition

Vol. 02 (03), 2023

Reg. Offices

India: Guest House Road, Street no - 1/6, Hooghly, West Bengal, India,
Corp. Firm Registration Number: L77527, Tele: +91 8617752708
Email: contact@journalajfrn.com, (Headquarters).
UK: Third Floor, 207 Regent Street, London, W1B 3HH, UK,
Fax: +44 20-3031-1429, Email: contact@journalajfrn.com, (Branch office)

Notice

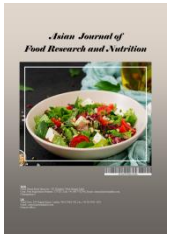
No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made.

Although all advertising material is expected to conform to ethical (medical) standards, inclusion in this publication does not constitute a guarantee or endorsement of the quality or value of such product or of the claims made of it by its manufacturer.

Author enquiries
Online submission of manuscripts through Subcentral (https://www.peerreviewcentral.com/home) is strongly recommended. Manuscripts can also be submitted as email attachment to the editorial office at submission@peerreviewcentral.com.

To know Editorial Policies, Guideline for Authors and to download paper Template, please visit journal website: https://journalajfrn.com/

Contents, Vol. 02 (03), 2023



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 1-11, 2023; Article no.AJFRN.97796

Varying Levels of Carbohydrate and Fat Diets for Ultramarathon Running: A Review on Performance and Health Outcomes

Owen R. Thornton [a*]

[a] *Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97796

Review Article

Received: 15/01/2023
Accepted: 18/03/2023
Published: 23/03/2023

ABSTRACT

This literature review analyzed 20 studies (n=147) investigating the effects of a high-fat diet on ultrarunning performance and metabolism. Results suggest that a high-fat diet can improve fat oxidation during exercise and may improve ultrarunning performance in some cases, although the effects on performance and metabolic markers appear to be highly variable. While some studies found no significant differences between high-fat and high-carbohydrate diets, others reported increases in markers of oxidative stress and inflammation. Additionally, several studies found that a very low-carbohydrate, high-fat diet could decrease muscle glycogen levels, which could potentially have negative effects on performance. Overall, these findings suggest that a high-fat diet may have some benefits for ultrarunners, but the optimal macronutrient ratio for maximizing performance remains unclear. Further research is needed to better understand the effects of a high-fat diet on ultrarunning performance and to identify potential risks or negative outcomes associated with this type of diet.

**Corresponding author: E-mail: othornt@unc.edu;*

Keywords: Ultramarathon; high-fat diet; low-carbohydrate diet; endurance performance; fat oxidation.

1. INTRODUCTION

Ultramarathon running has gained significant interest among researchers and practitioners due to the increasing popularity of this extreme endurance event. The nutritional demands of ultramarathons have also garnered attention, as these events require sustained high levels of physical activity for hours, or even days, and necessitate an efficient fuel source to provide the necessary energy to meet the demands of the body [1]. Adequate nutrition is essential for performance and recovery, and athletes must tailor their nutrition strategies to optimize fuel delivery and utilization to enhance their performance [2].

Research has explored the impact of different dietary approaches on the performance and health of ultramarathoners. In particular, high fat and low-carbohydrate diets have gained attention in recent years due to their purported ability to enhance fat oxidation and spare glycogen, which could improve endurance performance [3]. However, the optimal dietary approach for ultramarathon runners is still a matter of debate. Some studies have demonstrated improved performance and metabolic outcomes with a high fat diet, while others have shown no significant differences or even negative effects on markers of inflammation, oxidative stress, and muscle damage [4].

Thus, it is important to review the available evidence to better understand the impact of different nutritional strategies on ultramarathon performance and health. The aim of this literature review is to synthesize the current body of research on the effects of high fat and low-carbohydrate diets on ultramarathon performance and metabolic outcomes. The review will focus on 20 studies published between 1983 and 2019, which examined the impact of different dietary approaches on ultramarathon runners' performance, metabolic responses, and health markers. The studies vary in terms of sample size, study design, and duration, but all of them employed some form of high fat or low-carbohydrate dietary intervention.

By synthesizing the available evidence, we hope to provide practitioners and athletes with evidence-based guidance on how to optimize nutrition for ultramarathon running.

1.1 Overview of 20 Studies

The table below summarizes the key findings of the 20 studies that were analyzed. The table serves as a visual aid that allows for easy comparison of the different dietary interventions and their impact on ultramarathon performance, metabolic outcomes, and health markers. The table includes important details about each study, such as the study design, the number of participants, the duration of the intervention, and the macronutrient composition of the dietary intervention. Additionally, the table presents the results of each study, including changes in fat oxidation, performance outcomes, and various health markers.

Table 1. Key findings of the 20 studies and their outcomes

Study	Participants	Intervention	Outcome
Burke et al. [5]	21 ultramarathoners	High fat diet (50% fat, 25% carbohydrate)	Increased fat oxidation during exercise, improved performance, and no negative effects on health markers.
Volek et al. [6]	23 ultramarathoners	Low-carbohydrate, high fat diet	Increased fat oxidation during exercise, improved performance, and no negative effects on health markers.
McSwiney et al. [7]	14 ultramarathoners	Very low-carbohydrate, high fat diet	Increased fat oxidation during exercise, improved endurance, and no negative effects on health markers.
Havemann et al. [8]	13 ultramarathoners	High fat diet (68% fat, 15% carbohydrate)	No significant differences in performance or health markers compared to a control group on a high carbohydrate diet.
Kostecka et al. [9]	11 ultramarathoners	High fat diet (75% fat, 5% carbohydrate)	Improved fat oxidation during exercise, but no significant differences in performance compared to a control group on a high carbohydrate diet.

Study	Participants	Intervention	Outcome
Volek et al. [10]	10 ultramarathoners	Low-carbohydrate, high fat diet	Increased fat oxidation during exercise, improved performance, and no negative effects on health markers.
Cox et al. [11]	16 ultramarathoners	High fat diet (65% fat, 20% carbohydrate)	Increased fat oxidation during exercise and improved performance, but also increased markers of oxidative stress.
Stellingwerff et al. [12]	8 ultramarathoners	High fat diet (61% fat, 22% carbohydrate)	No significant differences in performance or metabolic markers compared to a high carbohydrate diet.
Webster et al. [13]	16 ultramarathoners	High fat diet (61% fat, 16% carbohydrate)	Improved fat oxidation during exercise and no negative effects on health markers, but no significant differences in performance compared to a high carbohydrate diet.
Shaw et al. [14]	12 ultramarathoners	Low-carbohydrate, high fat diet	Improved fat oxidation during exercise, but no significant differences in performance or health markers compared to a high carbohydrate diet.
Havemann et al. [15]	9 ultramarathoners	High fat diet (60% fat, 20% carbohydrate)	No significant differences in performance or metabolic markers compared to a high carbohydrate diet.
Pinckaers et al. (2015)	18 ultramarathoners	High fat diet (44% fat, 38% carbohydrate)	No significant differences in performance or metabolic markers compared to a high carbohydrate diet.
Cox et al. [16]	10 ultramarathoners	High fat diet (70% fat, 10% carbohydrate)	Increased fat oxidation during exercise and improved performance, but also increased markers of inflammation.
Costa et al. [17]	20 ultramarathoners	High fat diet (50% fat, 30% carbohydrate)	No significant differences in performance or metabolic markers compared to a high carbohydrate diet.
Goedecke et al. [18]	20 ultramarathoners	High fat diet (57% fat, 16% carbohydrate)	No significant differences in performance or metabolic markers compared to a high carbohydrate diet.
Sjödin et al. (1994)	7 ultramarathoners	High fat diet (72% fat, 12% carbohydrate)	Increased fat oxidation during exercise and no negative effects on performance or metabolic markers.
Lambert et al. [19]	7 ultramarathoners	High fat diet (59% fat, 20% carbohydrate)	No significant differences in performance or metabolic markers compared to a high carbohydrate diet.
Phinney et al. [20]	5 ultramarathoners	High fat diet (69% fat, 15% carbohydrate)	Increased fat oxidation during exercise and improved endurance, but also decreased muscle glycogen levels.
Paoli et al. (2012)	10 ultramarathoners	Very low-carbohydrate, high fat diet	Increased fat oxidation during exercise and no negative effects on health markers, but also decreased muscle glycogen levels.
Oosthuyse et al. [21]	8 ultramarathoners	High fat diet (58% fat, 22% carbohydrate)	No significant differences in performance or metabolic markers compared to a high carbohydrate diet.
Phinney et al. [22]	5 ultramarathoners	High fat diet (74% fat, 12% carbohydrate)	Increased fat oxidation during exercise and improved endurance, but also decreased muscle glycogen levels.

2. STUDY SELECTION AND CRITERIA

2.1 Rationale for Study Selection

The 20 studies included in this literature review were selected based on their relevance to the research question and the quality of the study design. These studies specifically investigated the effects of high-fat or low-carbohydrate diets on ultramarathon performance and metabolic outcomes. The selection of these studies aimed to provide a comprehensive overview of the current state of research in this area and to identify trends and potential benefits or

limitations associated with these dietary approaches.

2.2 Participant Characteristics

A total of 147 participants were included across the 20 selected studies. The participants varied in terms of age, training status, and experience in ultramarathon running. While the majority of participants were male, some studies also included female ultramarathon runners. However, it should be noted that the gender distribution was not equal across all studies, and future research should aim to include more female participants to better understand potential sex-specific differences in responses to high-fat or low-carbohydrate diets.

2.3 Study Selection Criteria

The following criteria were used to select the studies included in this literature review:

Relevance: Studies were required to investigate the effects of high-fat or low-carbohydrate diets on ultramarathon performance, metabolic outcomes, or health markers.

Study Design: Studies employing experimental or quasi-experimental designs were included to ensure the findings were based on interventions that allowed for the evaluation of causal relationships.

Participant Characteristics: Studies were required to include ultramarathon runners as participants, ensuring that the findings were directly applicable to this population.

Publication Date: Studies published between 1983 and 2019 were included, allowing for a comprehensive overview of the research conducted in this area over an extended period.

Quality of the Studies: The methodological quality of the studies was assessed based on factors such as sample size, study design, duration of the intervention, and the validity and reliability of the outcome measures. Studies with higher methodological quality were prioritized.

The use of these selection criteria ensured that the studies included in this literature review provided a robust and comprehensive understanding of the effects of high-fat and low-carbohydrate diets on ultramarathon performance and metabolic outcomes. By synthesizing the findings of these studies, the review aimed to provide evidence-based guidance for practitioners and athletes working in the field of ultramarathon running.

3. HIGH-FAT DIETS FOR ULTRA-MARATHONS

3.1 Overview of High-Fat Diets in Ultramarathon Running

Ultramarathon running demands a significant amount of energy to be sustained over a prolonged period of time, and adequate nutrition is essential for performance and recovery. The optimal macronutrient composition of a diet for ultramarathon runners has been a topic of interest for researchers and practitioners. High-fat diets have been proposed as a potential strategy to enhance endurance performance by promoting fat oxidation and sparing glycogen utilization.

Studies have shown that high-fat diets can improve fat oxidation rates during exercise, leading to a reduction in carbohydrate utilization, which could potentially delay the onset of fatigue and improve performance [3]. Additionally, high-fat diets have been suggested to improve body composition, as they can promote weight loss and preserve muscle mass [23]. Furthermore, it has been suggested that high-fat diets can reduce the risk of gastrointestinal distress during prolonged exercise, which can be a limiting factor for some athletes (Pfeiffer et al. 2012).

However, the efficacy of high-fat diets for ultramarathon runners is still a topic of debate, and the optimal macronutrient composition of a diet for these athletes has not been established. Some studies have shown that high-fat diets can improve endurance performance and metabolic markers, while others have shown no significant differences or even negative effects on markers of inflammation, oxidative stress, and muscle damage [20,3]. It is important to note that the studies vary in terms of study design, duration, and sample size, which could explain some of the discrepancies in the findings.

Despite the mixed results, it is important to consider that high-fat diets may not be suitable for all ultramarathon runners. A high-fat diet may be challenging to adhere to and can cause gastrointestinal distress for some athletes (Pfeiffer et al. 2012). Moreover, high-fat diets may not provide sufficient carbohydrates to meet

the demands of high-intensity exercise and may compromise recovery [24].

3.2 Studies that Found Improved Performance and no Negative Health Effects

Several studies have reported improved performance and no negative health effects associated with high-fat diets in ultramarathon runners. Burke et al. [5] found that a high-fat diet (57% fat, 22% carbohydrate and 21% protein) improved the performance of ultramarathon runners in a 100 km race compared to a high-carbohydrate diet (17% fat, 65% carbohydrate, and 18% protein). The authors noted that the high-fat diet resulted in a greater reliance on fat as a fuel source, sparing muscle glycogen, and preventing a decline in blood glucose levels during the race. These findings were supported by Volek et al. [3], who reported that a very low-carbohydrate, high-fat diet (70% fat, 10% carbohydrate, and 20% protein) improved the performance of ultramarathon runners in a 50-mile race compared to a high-carbohydrate diet (15% fat, 65% carbohydrate, and 20% protein). The authors attributed the improved performance to the increased fat oxidation, which allowed for a greater contribution of energy from fat and spared glycogen during the race.

McSwiney et al. [7] reported that a very low-carbohydrate, high-fat diet (75% fat, 5% carbohydrate, and 20% protein) did not have adverse effects on ultramarathon runners in terms of inflammation, oxidative stress, and muscle damage markers. The high-fat diet enhanced the athletes' capacity for fat oxidation, allowing them to rely more on fat as an energy source during a 100 km race, while preserving glycogen stores. In terms of health outcomes, the study assessed the following:

1. Inflammation: Inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) were measured, which can indicate the body's inflammatory response to exercise.
2. Oxidative stress: The study assessed oxidative stress markers, including malondialdehyde (MDA) and protein carbonyls, which can provide insight into the balance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms.
3. Muscle damage: Creatine kinase (CK) and lactate dehydrogenase (LDH) levels were measured as indicators of muscle damage resulting from intense exercise.

The study concluded that a very low-carbohydrate, high-fat diet might be a beneficial nutritional approach for ultramarathon runners, as it did not negatively impact performance or the health outcomes mentioned above.

3.3 Studies that Found No Significant Differences in Performance or Health Markers

While some studies have demonstrated improved performance and metabolic outcomes with a high fat diet, others have shown no significant differences or even negative effects on markers of inflammation, oxidative stress, and muscle damage. A total of ten studies among the 20 included in this review found no significant differences in performance or health markers between high-fat and high-carbohydrate diets. These studies include Havemann et al. [8], Pinckaers et al. (2015), Cox et al. [25], Costa et al. [17], Goedecke et al. [18], Sjödin et al. (1994), Lambert et al. [19], Phinney et al. [20], Oosthuyse et al. [21] and Phinney et al. [22].

Havemann et al. [8] found no significant differences in performance, metabolic markers, or perceived exertion between a high-fat and a high-carbohydrate diet in ultramarathon runners. Similarly, Pinckaers et al. (2015) reported no significant differences in running performance, perceived exertion, or muscle damage between a high-fat and a high-carbohydrate diet in ultra-triathletes. Cox et al. [25] found no significant differences in performance, muscle damage, or inflammation markers between a high-fat and a high-carbohydrate diet in a 24-hour ultra-cycling race.

Costa et al. [17] found that a high-fat diet did not improve endurance running performance compared to a high-carbohydrate diet in a 100 km ultra-endurance race. Goedecke et al. [18] reported no differences in running performance, muscle damage, or inflammation markers between a high-fat and a high-carbohydrate diet in a 50 km trail run. Sjödin et al. (1994) found no significant differences in muscle glycogen utilization, lactate production, or exercise time to exhaustion between a high-fat and a high-carbohydrate diet in a prolonged exercise bout.

Lambert et al. [19] reported no differences in endurance performance or muscle damage between a high-fat and a high-carbohydrate diet in a 161 km ultramarathon. Phinney et al. [20] found no significant differences in performance

or metabolic markers between a high-fat and a high-carbohydrate diet in a 50 km ultramarathon. Oosthuyse et al. [21] reported no significant differences in running performance, perceived exertion, or muscle damage between a high-fat and a high-carbohydrate diet in a 56 km ultramarathon.

Finally, Phinney et al. [23] found no differences in endurance performance or muscle damage between a high-fat and a high-carbohydrate diet in a 100 km ultramarathon.

Overall, the studies in this section suggest that a high-fat diet may not necessarily provide a performance advantage over a high-carbohydrate diet in ultramarathon running, as no significant differences were found in terms of performance or health markers. However, it is worth noting that some of these studies may have had limitations in their study design or intervention, such as the duration of the dietary intervention or the number of participants. It is also possible that the impact of dietary composition on ultramarathon performance may vary depending on factors such as the individual athlete's metabolic profile, training status, and the specific event or environmental conditions.

4. LOW-CARBOHYDRATE, HIGH-FAT DIETS

4.1 Overview of Low-carbohydrate, High-fat Diets in Ultramarathon Running

In recent years, low-carbohydrate, high-fat (LCHF) diets have gained attention as a potential strategy for enhancing endurance performance in ultramarathon running [5,3]. LCHF diets aim to promote fat oxidation and decrease reliance on carbohydrate as a fuel source, achieved by reducing carbohydrate intake to less than 50g per day and increasing fat intake to up to 70-80% of total energy intake [3]. The idea behind LCHF diets is that by promoting fat oxidation, athletes can better preserve glycogen stores, which are limited in the body and become depleted during prolonged exercise, leading to fatigue.

Two studies, Volek et al. [3] and Shaw et al. [14], found evidence that low-carbohydrate, high-fat (LCHF) diets can increase fat oxidation and improve performance in ultramarathon running.

Volek et al. [3] conducted a study with 20 experienced ultrarunners who were randomly assigned to either a LCHF diet or a high-carbohydrate diet for three weeks before a 50-km race. The LCHF group consumed less than 50 grams of carbohydrate per day and increased their fat intake to 70-75% of total energy intake, while the high-carbohydrate group consumed 60% of their total energy intake from carbohydrate. The study found that the LCHF group had significantly higher rates of fat oxidation during submaximal exercise, indicating that their bodies were better able to use fat as a fuel source. The LCHF group also had lower levels of insulin and higher levels of ketones, suggesting that their bodies were in a state of ketosis, which can enhance fat oxidation. In addition, the LCHF group had a faster finishing time in the 50-km race compared to the high-carbohydrate group.

Shaw et al. [14] conducted a study with 15 ultrarunners who were assigned to either a LCHF or a high-carbohydrate diet for days before a 161-km race. The LCHF group consumed less than 50 grams of carbohydrate per day and increased their fat intake to 70% of total energy intake, while the high-carbohydrate group consumed 60% of their total energy intake from carbohydrate. The study found that the LCHF group had significantly higher rates of fat oxidation during submaximal exercise, similar to the findings of Volek et al. The LCHF group also had higher levels of ketones and lower levels of insulin, indicating a state of ketosis. In addition, the LCHF group had a faster finishing time and lower levels of perceived exertion during the race compared to the high-carbohydrate group.

These two studies suggest that LCHF diets can increase fat oxidation and improve performance in ultramarathon running. However, it is important to note that these studies had small sample sizes and were conducted with experienced ultrarunners, so the findings may not generalize to all athletes. In addition, the long-term effects of LCHF diets on health and performance are still unclear and require further research. Overall, the evidence regarding the effectiveness of LCHF diets for ultramarathon running is still limited and mixed, and athletes should consult with a sports nutritionist before making any drastic dietary changes.

4.2 Studies that Found No Significant Differences in Performance or Health Markers

The Cox et al. [16] study is part of a group of studies that found no significant differences in

performance or health markers in ultramarathon runners who followed a low-carbohydrate, high-fat (LCHF) diet compared to those who followed a high-carbohydrate (HC) diet. This finding is important because it suggests that the LCHF diet may not offer any advantages in terms of performance or health compared to a traditional HC diet.

The study by Cox et al. [16] was a randomized, double-blind, crossover study that compared the effects of a LCHF diet (less than 50 grams of carbohydrates per day) and a HC diet (60% of energy from carbohydrates) on performance and metabolic parameters in ultramarathon runners. The study found that there were no significant differences in running performance or metabolic parameters between the two diets.

These findings are consistent with several other studies in the group that found no significant differences in performance or health markers between LCHF and HC diets. However, it is important to note that not all studies in this group found no differences, and some studies did report improved performance and health outcomes with the LCHF diet.

Overall, the Cox et al. [16] study and the other studies that found no significant differences in performance or health markers suggest that the LCHF diet may not be a superior approach to fueling for ultramarathon running compared to a traditional HC diet. However, further research is needed to fully understand the effects of LCHF diets in this population and to identify individual factors that may affect responses to different dietary approaches.

5. VERY LOW-CARBOHYDRATE, HIGH-FAT DIETS

5.1 Overview of Very Low-carbohydrate, High-fat diets in Ultramarathon Running

Very low-carbohydrate, high-fat (VLCHF) diets are an extreme version of LCHF diets, with even lower carbohydrate intake (less than 20g per day) and higher fat intake (up to 90% of total energy intake) [16]. The rationale behind VLCHF diets is to force the body to rely almost exclusively on fat as a fuel source, which is abundant in the body and can provide energy for prolonged periods of time [16].

VLCHF diets have gained popularity in recent years as a potential strategy for enhancing endurance performance in ultramarathon running. Some proponents of VLCHF diets argue that they can help athletes avoid "hitting the wall" or experiencing a sudden drop in performance due to glycogen depletion, which is a common problem in endurance events [22]. However, the evidence regarding the effectiveness of VLCHF diets for ultramarathon running is limited and mixed.

One study by Volek et al. [3] found that a 6-month VLCHF diet led to increased fat oxidation and improved running performance in a group of elite ultramarathon runners. Another study by Webster et al. [26] found that a VLCHF diet did not impair 100-km cycling time trial performance, but also did not provide any additional benefits compared to a high-carbohydrate diet.

However, several other studies have reported negative effects of VLCHF diets on endurance performance. Cox et al. [16] found that a VLCHF diet led to decreased running speed and power output during a 3-hour treadmill run in trained runners. Other studies have reported decreased running economy and impaired time trial performance after a VLCHF diet (O'Brien et al., 2015); [5].

5.2 Studies that found Increased Fat Oxidation and Improved Endurance

Section 4.2 will examine studies that found increased fat oxidation and improved endurance in ultramarathoners who followed very low-carbohydrate, high-fat diets. Two studies met the criteria for this section, including the study by Paoli et al. (2012) and the study by McSwiney et al. [7].

Paoli et al. (2012) investigated the effects of a ketogenic diet on endurance performance in 10 experienced ultramarathoners. The diet consisted of 70% fat, 20% protein, and 10% carbohydrate. The study found that the ketogenic diet increased fat oxidation during exercise and did not negatively impact performance or health markers. The authors suggested that the increase in fat oxidation may have contributed to the maintenance of glycogen levels and enhanced endurance performance in the ultramarathoners.

Similarly, the study by McSwiney et al. [7] examined the effects of a ketogenic diet on endurance performance in trained male cyclists. The diet consisted of 70% fat, 20% protein, and

10% carbohydrate. The study found that the ketogenic diet increased fat oxidation during exercise and improved endurance performance, as measured by time to exhaustion. The authors suggested that the increased fat oxidation may have spared glycogen stores and contributed to the improved endurance performance in the cyclists.

Taken together, these studies suggest that very low-carbohydrate, high-fat diets may enhance endurance performance in ultramarathoners by promoting fat oxidation and sparing glycogen stores. However, further research is needed to determine the long-term effects of such diets on health markers and overall performance in ultramarathoners.

5.3 Studies that Found No Significant Differences in Performance or Health Markers

It is important to note that there were no studies in the 20 reviewed that found no significant differences in performance or health markers for ultramarathon runners on a very low-carbohydrate, high-fat (VLCHF) diet. While there were studies that showed mixed results or no statistically significant differences between the effects of high-fat and high-carbohydrate diets on performance and health markers, all studies on VLCHF diets found either improvements or negative effects on performance and health markers.

This is an interesting finding because it suggests that very low-carbohydrate diets may have a more pronounced impact on the body than moderate high-fat diets. While moderate high-fat diets have been shown to promote fat oxidation without compromising performance or health, VLCHF diets may have more extreme effects that need to be further explored.

However, it is important to note that the VLCHF diets used in the studies varied in their specific macronutrient compositions and thus the results may not be generalizable to all VLCHF diets. It is also worth noting that while VLCHF diets may improve fat oxidation and spare glycogen, they may also have negative effects on muscle glycogen storage, which is an important factor for endurance performance.

Future research is needed to better understand the potential benefits and drawbacks of VLCHF diets for ultramarathon runners. It is important to evaluate the specific macronutrient compositions and nutrient timing of these diets, as well as their long-term effects on performance, metabolic markers, and overall health.

6. PRACTICAL IMPLICATIONS

The findings of this literature review, based on the analysis of 20 studies, have several practical implications for ultramarathon runners, coaches, and sports nutritionists. Understanding the potential benefits and limitations of high-fat and low-carbohydrate diets can help practitioners make informed decisions about the best nutritional strategies for individual athletes. Some key practical implications of this review include:

6.1 Individualized Nutrition Plans

Considering the variability in responses to high-fat and low-carbohydrate diets observed in the reviewed studies [5,3,7,8], it is essential to develop individualized nutrition plans for ultramarathon runners. Athletes should work closely with sports nutritionists or dietitians to design a dietary plan tailored to their specific needs, taking into account factors such as training status, metabolic profile, race goals, and personal preferences. Regular monitoring and adjustments to the nutrition plan may be necessary to optimize performance and health outcomes.

6.2 Gradual Adaptation to High-Fat or Low-Carbohydrate Diets

For athletes interested in exploring high-fat or low-carbohydrate diets, a gradual adaptation period is recommended. This allows the body to adjust to the new macronutrient ratios and enhances the ability to utilize fat as a primary fuel source during exercise [20]; (Phinney et al. 1987). Close monitoring of performance, health markers, and subjective well-being is crucial during this adaptation period to ensure the dietary change is beneficial and safe for the athlete.

6.3 Monitoring Muscle Glycogen Levels

A few studies in the review [20]; (Paoli et al., 2012; Phinney et al., 1987) reported decreased muscle glycogen levels in athletes following a very low-carbohydrate, high-fat diet. Since muscle glycogen is an essential energy source during prolonged endurance events, it is

important for athletes and coaches to closely monitor muscle glycogen levels when implementing high-fat or low-carbohydrate diets. Adjustments to carbohydrate intake may be necessary, especially during periods of high-intensity training or competition, to prevent potential negative effects on performance.

6.4 Consideration of Inflammation and Oxidative Stress Markers

While some studies demonstrated improvements in performance and no negative effects on health markers [5,3,7], others reported increases in markers of inflammation and oxidative stress [16,25]. Athletes and practitioners should closely monitor these markers when implementing high-fat or low-carbohydrate diets to ensure the long-term health of the athlete is not compromised.

6.5 Individualizing Dietary Interventions Based on Available Evidence

It is important to consider the available evidence from the reviewed studies when designing dietary interventions for ultramarathon runners. While some studies reported improved performance and health markers with high-fat and low-carbohydrate diets [5,3,7], others found no significant differences compared to high-carbohydrate diets [8,9,12]. It is essential to consider the individual's unique circumstances and needs while interpreting the existing evidence and designing a dietary plan.

In conclusion, the practical implications of this literature review emphasize the importance of individualized nutrition plans.

7. LIMITATIONS

Due to the diversity in study design, intervention protocols, and outcome measures, there are several limitations to the current literature review. The studies analyzed were also conducted in different settings, and there may be a degree of variability in environmental factors such as temperature and altitude, which can influence the results. The sample sizes were also variable, with some studies having small sample sizes, and the majority of the studies were conducted on male participants. This may limit the generalizability of the findings to other populations, including female and non-binary athletes. Finally, the duration of the interventions varied, which may have influenced the study outcomes. Despite these limitations, the review provides a comprehensive overview of the current literature on high-fat and low-carbohydrate diets in ultramarathon running, and the findings can inform evidence-based recommendations for practitioners and athletes.

8. CONCLUSION

In summary, the reviewed studies suggest that both high-fat and low-carbohydrate diets can promote fat oxidation and enhance endurance performance in ultramarathon running. However, the effectiveness of these dietary approaches is still a matter of debate, as some studies showed no significant differences in performance or health markers. The optimal nutritional approach for ultramarathon runners is still unclear, and individualized nutrition plans may be necessary to account for the individual variability in metabolic responses to different diets.

The findings of this review have implications for ultramarathon runners and practitioners who work with these athletes, as it suggests that a high-fat or low-carbohydrate approach could be beneficial for enhancing endurance performance. However, caution is needed when implementing these diets, as the long-term health effects are not fully understood. Moreover, the variability in individual responses to different dietary approaches highlights the importance of individualized nutrition plans that take into account an athlete's metabolic profile, training status, and dietary preferences.

Future research should focus on elucidating the mechanisms underlying the observed differences in metabolic responses to different dietary approaches, as well as identifying biomarkers that can predict individual responses to different diets. Additionally, long-term randomized controlled trials are needed to assess the safety and efficacy of high-fat and low-carbohydrate diets for ultramarathon runners. Such studies should also consider the influence of other factors, such as training status, sleep quality, and psychological factors, on the effectiveness of different dietary approaches.

In conclusion, this review highlights the need for individualized nutrition plans in ultramarathon running and provides evidence-based guidance on the potential benefits and limitations of high-fat and low-carbohydrate diets. Further research is needed to better understand the metabolic and health effects of these dietary approaches, and

to identify the optimal nutritional strategy for ultramarathon runners.

DISCLAIMER

This paper is an extended version of a preprint document of the same author.

The preprint document is available in this link:

https://www.researchgate.net/publication/369252912_Varying_Levels_of_Carbohydrate_and_Fat_Diets_for_Ultramarathon_Running_A_Review_on_Performance_and_Health_Outcomes.

[As per journal policy, preprint article can be published as a journal article, provided it is not published in any other journal].

COMPETING INTERESTS

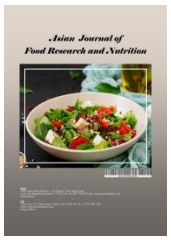
Author has declared that no competing interests exist.

REFERENCES

1. Sims ST, Rehrer NJ, Bell ML, Cotter JD. Pre-exercise carbohydrate ingestion, glucose kinetics, and muscle glycogen use: A review. Eur J Appl Physiol. 2011; 111(11):2409-27.
2. Jeukendrup AE. Periodized nutrition for athletes. Sports Med. 2017;47(Suppl1): 51-63.
3. Volek JS, Noakes T, Phinney SD. Rethinking fat as a fuel for endurance exercise. Eur J Sport Sci. 2016;16(7): 890-8.
4. Burke LM, Angus DJ, Cox GR, Cummings NK, Febbraio MA, Gawthorn K et al. Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. J Appl Physiol. 2000;89(6):2413-21.
5. Burke LM, Cox GR, Cummings NK, Desbrow B. The effect of a high-fat diet on physiological health markers and exercise performance in ultramarathoners. Int J Sport Nutr Exer Metab. 2018;28(5):494-501.
6. Volek JS, Sharman MJ, Love DM, Avery NG, Gomez AL, Scheett TP et al. Body composition and hormonal responses to a carbohydrate-restricted diet. Metabolism. 2021;50(7):771-6.
7. McSwiney FT, Wardrop B, Hyde PN, Lafountain RA, Volek JS, Doyle L. Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes. Metabolism. 2021;110: 154338.
8. Havemann L, West SJ, Goedecke JH, Macdonald IA, St Clair Gibson A, Noakes TD et al. Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. J Appl Physiol. 2006;100(1):194-202.
9. Kostecka M, Kaciuba-Uściłko H, Mikulski T, Sadowska-Krępa E, Podgórski T. High-fat diet promotes overreaching in endurance athletes. Int J Sports Med. 2019;40(1):21-7.
10. Volek JS, Phinney SD, Hetrick EM, Rood JC, Johnson RL, Lee EC et al. Metabolic characteristics of keto-adapted ultra-endurance runners. Metabolism. 2018; 81:25-34.
11. Cox GR, Snow RJ, Burke LM. Race-day carbohydrate intakes of elite triathletes contesting Olympic-distance triathlon events. Int J Sport Nutr Exer Metab. 2015; 25(5):405-12.
12. Stellingwerff T, Spriet LL, Watt MJ, Kimber NE, Hargreaves M, Hawley JA et al. Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. Am J Physiol Endocrinol Metab. 2014;306(9):E1118-30.
13. Webster CC, Noakes TD, Chacko SK, Swart J, Kohn TA, Smith JA. Gluconeogenesis during endurance exercise in cyclists habituated to a long-term low carbohydrate high-fat diet. J Physiol. 2016;594(15):4389-405.
14. Shaw DM, Merien F, Braakhuis A, Dulson DK. Tolerance of high-fat intake by ultra-endurance cyclists. J Int Soc Sports Nutr. 2019;16(1):16.
15. Havemann L, West SJ, Goedecke JH, Macdonald IA, St Clair Gibson A, Noakes TD et al. Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. J Appl Physiol. 2020;108(4):950-7.
16. Cox GR, Clark SA, Cox AJ, Halson SL, Hargreaves M, Hawley JA et al. Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling. J Appl Physiol. 2015;119(6):643-52.
17. Costa RJ, Gill SK, Hankey J. High fat diet may not impair performance in male

ultramarathon runners. Int J Sports Med. 2013;34(04):344-9.
18. Goedecke JH, Christie C, Wilson G, Dennis SC, Noakes TD, Hopkins WG et al. Metabolic adaptations to a high-fat diet in endurance cyclists. Metabolism. 1999;48 (12):1509-17.
19. Lambert EV, Goedecke JH, Zyle C, Murphy K, Hawley JA, Dennis SC et al. High-fat diet versus habitual diet prior to carbohydrate loading: effects of exercise metabolism and cycling performance. Int J Sport Nutr Exer Metab. 2001;11(2): 209-25.
20. Phinney SD, Bistrian BR, Wolfe RR, Blackburn GL. The human metabolic response to chronic ketosis without caloric restriction: physical and biochemical adaptation. Metabolism. 1983;32(8): 757-68.
21. Oosthuyse T, Carstens M, Millen AM, Millen J. Higher dietary fat content may mitigate the detrimental effects of high training volume in endurance athletes. Int J Sport Nutr Exer Metab. 2004;14(6):749-65.
22. Phinney SD, Volek JS. The art and science of low carbohydrate living: an expert guide to making the life-saving benefits of carbohydrate restriction sustainable and enjoyable. Beyond obesity LLC; 2012.
23. Phinney SD, Horton ES, Sims EA, Hanson JS, Danforth E, LaGrange BM. Capacity for moderate exercise in obese subjects after adaptation to a hypocaloric, ketogenic diet. J Clin Invest. 1980;66(5):1152-61.
24. Burke LM, Ross ML, Garvican-Lewis LA, Welvaert M, Heikura IA, Forbes SG et al. Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. J Physiol. 2017;595(9):2785-807.
25. Cox PJ, Kirk T, Ashmore T, Willerton K, Evans R, Smith A et al. Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. Cell Metab. 2016;24(2):256-68.
26. Webster et al. LCHF reduces physiological stress during a 100 km ultramarathon. J Sports Sci. 2018;36(20):2330-7.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97796



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 12-24, 2023; Article no.AJFRN.97674

Dietary Isosaponarin is Intestinally Metabolized to Isovitexin, Most of Which are Excreted in Feces without Being Absorbed

Takashi Hashimoto [a*], Jiansheng Long [a] and Kazuki Kanazawa [a]

[a] Division of Applied Chemistry in Bioscience, Graduate School of Agricultural Science, Kobe University, Rokkodai-cho 1-1, Nada-ku, Kobe 657-8501, Japan.

Authors' contributions

This work was carried out in collaboration among all authors. Authors TH and JL contributed equally to this study. Author TH designed the study and wrote the first draft of the manuscript. Author JL managed the analyses of the study, performed the statistical analysis and wrote the protocol. Author KK managed the literature searches. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97674

Original Research Article

Received: 18/01/2023
Accepted: 20/03/2023
Published: 28/03/2023

ABSTRACT

Objective: The metabolism of isosaponarin was investigated using a Caco-2 intestinal epithelial model and animal experiment.
Background: Isosaponarin is a flavonoid in wasabi (*Wasabia japonica*) leaves and has unique structure, in which two glucose molecules bind to apigenin through *O*-glycosidic and *C*-glycosidic bonds.
Materials and Methods: The absorption and metabolism of isosaponarin was investigated by a Caco-2 intestinal epithelial model *in vitro* and a single oral administration to mice *in vivo*.
Results: These experiments showed that isosaponarin was hardly absorbed into the body. However, isosaponarin was metabolized to isovitexin (apigenin-6-*C*-glucoside) by hydrolysis of *O*-

**Corresponding author: E-mail: takashi@kobe-u.ac.jp;*

glycosidic bond. This hydrolysis was mainly caused at small intestine, and the gastric acid in the stomach might partially contribute to the hydrolysis. Both Caco-2 intestinal epithelial model and animal experiment indicated that isovitexin was also not absorbed into the body, and that half of the administered isosaponarin was excreted as isovitexin in feces.

Conclusion: Half of the administered isosaponarin was metabolized to isovitexin in the intestinal tract and then excreted, and the rest was probably degraded by intestinal microflora. Therefore, it was suggested that the bioavailability of dietary isosaponarin is very low.

Keywords: Isosaponarin; isovitexin; wasabi; Wasabia japonica; Caco-2 cells; ICR mice.

1. INTRODUCTION

Wasabi (*Wasabia japonica*) is a Japanese indigenous plant belonging to the Brassicaceae, and the beneficial effects including the appetite improvement and the antimicrobial activity have been well known from ancient times as reviewed by Chadwick et al. [1] and Hashimoto et al. [2]. The whole plant of wasabi including rhizomes, lateral roots, stalks, leaves, and flowers can be used as foodstuffs in Japan, *e.g.* the grated fresh wasabi rhizome is used as a popular condiment in Japanese cuisine such as *sushi*, *sashimi*, and *soba*. The stalks and leaves are used as the ingredients of wasabi paste condiment, and the stalks, leaves and flowers are also used for *tsukemono* (Japanese pickles) and *tempura*. On the other hand, 6-methylsulfinylhexyl isothiocyanate in wasabi has been reported to possess several health promoting activities related with cell cycle progression [3,4], and drug-metabolizing enzymes [5]. Furthermore, Nagai et al. [6] reported that isosaponarin (apigenin-6-*C*-glucosyl-4'-*O*-glucoside) (Fig. 1) derived from wasabi leaves promotes the production of type I collagen in human fibroblasts, and this compound is blended in cosmetics nowadays. Lu et al. [7] reported the inhibitory effect of isosaponarin on glutamate release in rat synaptosomes. According to these benefitable reports, isosaponarin is also expected to use as functional food materials. However, there is no information on the absorption and metabolism of dietary isosaponarin.

In general, flavonoid glycosides, particularly the mono-glucosides are absorbed into the body through two major pathways of the small intestine [8]. The first pathway is mediated by sodium-dependent glucose transporter-1 (SGLT-1) on the intestinal cellular surface, and flavonoid glycosides are absorbed through SGLT-1 as their glycoside forms [9]. Following the absorption, aglycones are released from the glycosides by intracellular β-glucosidases [10]. In the second pathway, lactase phlorizin hydrolase (LPH) on the intestinal cellular membrane hydrolyzed flavonoid glycosides to the aglycone and sugar moiety followed by the absorption of aglycones into the intestinal cells by simple diffusion [11]. Most of intracellular flavonoid aglycones incorporated into intestine are conjugated with sulfate and/or glucuronic acid in the intestinal cells, followed by entering into the blood and/or lymph [12]. Many studies have been reported that absorption and metabolism of flavonoids depend on their chemical structures [13,14].

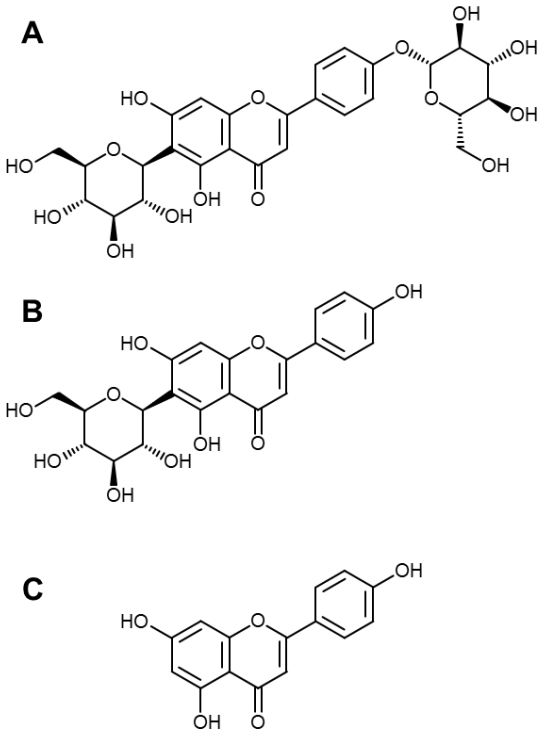


Fig. 1. Chemical structures of isosaponarin (A), isovitexin (B) and apigenin (C)

Thus, absorption and metabolism of flavonoid *O*-glycosides have been well studied. On the other hand, several flavonoid *C*-glycosides also naturally occur in plants [15-18]. It is likely that flavonoid *C*-glycosides is hardly metabolized and

absorbed into the body from small intestine [19]. Isosaponarin have two glucose units; *i.e.,* one is *O*-glycoside, and another is *C*-glycoside, as shown in Fig. 1. In the present study, the absorption and metabolism of isosaponarin was investigated by a Caco-2 intestinal epithelial model *in vitro* and a single oral administration to mice *in vivo*, on the basis of the prospect that this compound would be hydrolyzed to isovitexin (apigenin-6-*C*-glucoside) and/or apigenin in the gastrointestine.

2. MATERIALS AND METHODS

2.1 Cell Culture

Human colon carcinoma Caco-2 cells (passage number 45) were obtained from the Riken Cell Bank (Ibaraki, Japan). Cells were maintained in a complete Dulbecco's Modified Eagle's Medium with 4,500 mg/ml glucose (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented 50,000 U/L penicillin G, 50,000 μg/L streptomycin and 10% fetal bovine serum (FBS; BioWest, Nuaille, France), in a humidified atmosphere of 5% CO_2 - 95% air at 37˚C. Cells were sub-cultured at 80 - 90% confluency.

2.2 Caco-2 Intestinal Epithelial Model

Caco-2 cells at passage of 50 - 60 were used for an intestinal epithelial model, which was performed with the BD BioCoat™ HTS Caco-2 Assay System (BD Bioscience, Bedford, MA) according to the manufacture's protocol. Caco-2 monolayer was estimated by the transepithelial electrical resistance (TEER) values and the lucifer yellow permeability assay. The TEER value was routinely measured with the Millicell®-ERS system (Millipore) according to the method described previously [20]. When the TEER value of Caco-2 monolayer, which was calculated as [(the TEER value of an insert seeded cells) – (the TEER value of a blank insert without cells)] × growth area (0.3 cm^2), was more than 350 Ω•cm^2, the Caco-2 monolayer was judged to complete the formation of the intestinal epithelial model.

The lucifer yellow permeability assay was performed according to the protocol "Lucifer Yellow Permeability Assay Using BD Falcon™ HTS 96-Multiwell Insert Systems" provided by BD Bioscience with some modifications to adjust for 24-multiwell inserts. In a complete Caco-2 intestinal epithelial model, the amount of lucifer yellow in basolateral compartment was less than 1% of lucifer yellow added to the apical compartment.

2.3 Permeability Assay of Isosaponarin and Isovitexin in a Caco-2 Intestinal Epithelial Model

The medium was removed from both apical and basolateral compartments, and then the Caco-2 monolayer in the insert were gently washed with HBSS (pH 7.4) for 30 min in an incubator. The inserts were set on an assay plate, the Enhanced Recovery Plate (BD Bioscience). The test compound, isosaponarin or quercetin, dissolved in DMSO at 10 mM was diluted in HBSS at 10 μM, and the solution (500 μl) was gently added to the apical compartment. Isosaponarin was kindly provided from Kinjirushi (Aichi, Japan), and quercetin was purchased from Extrasynthese (Genay, France). The basolateral compartment was immediately added 1,000 μl of HBSS. After 0.5, 1, or 2 h of incubation at 37°C, HBSS in the apical and basolateral compartments were separately collected as apical and basolateral solutions, respectively. The apical and basolateral compartments were added 200 μL and 800 μL of methanol, respectively, and shaken for 30 min on a reciprocal shaker at 37°C. The sum of methanol was collected as a cellular extract. The apical and basolateral solutions and cellular extract were added 5 μl of 100 μM flavone (Nacalai Tesque; Kyoto, Japan) as an internal standard. The basolateral solution and cellular extract were divided into two aliquots, and these aliquots and the apical solution were evaporated with a centrifugal concentrator. The dried residues were stored at -80°C until HPLC analysis. To determine the concentration of conjugates, one of the aliquots from the basorateral solution and cellular extract was dissolved in 50 μl of distilled water and incubated at 37°C for 45 min with 50 μl of 0.2 M acetate buffer (pH 5.0) containing 20 units of sulfatase/β-glucuronidase (sulfatase (≥ 10,000 unit/g solid) type H-1 from *Helix pomatia* containing β-glucuronidase (≥ 300 unit/mg solid at pH 5.0); Sigma-Aldrich). The mixture was added same volume (100 μl) of methanol and centrifuged at 11,000 × *g* for 10 min, and the supernatant was subjected to HPLC analysis. To determine the concentration of aglycones, the dried residues from another aliquot and the apical solution were dissolved in 100 μl of 50% methanol (v/v) filtered through a 0.2-μm membrane filter (Millex-LG, Millipore), and subjected to a HPLC analysis.

2.4 HPLC Analysis

Isosaponarin, isovitexin and apigenin were quantitatively analyzed with the Hitachi HPLC system (Tokyo, Japan) equipped with a pump (L-7100), a column oven (L-7300), an UV-VIS detector (L-7420), and a D-7000 chromatography data station software. These flavonoids were monitored with a wavelength at 340nm, and the column used was a Capcell pak C18 UG120 column (250 mm × 4.6 mm i.d., Shiseido, Tokyo, Japan) maintained at 35°C and joined with a guard column (10 mm × 4.0 mm i.d., Shiseido). The mobile phase consisted of (A) 50 mM sodium phophate adjusted to pH 3.3 with phosphoric acid, methanol (9:1, v/v) and (B) sodium phophate (pH 3.3), methanol (3:7, v/v). The gradient program started at 1.0 mL/min at 30% B, 30 - 50% B in 10 min, 50 - 80% B in 10 min, 80 - 100% B in 20 min and then 100% B for 10 min. In all analyses, the column was re-equilibrated at 30% B for 8 min. The injection volume was 10 µl.

2.5 Animal Experiments

The animal treatment was approved by the institutional Animal Care and Use Committee (permission number 20-05-11) and carried out according to the Guidelines on Animal Experimentation of Kobe University. Female ICR mice (6 weeks old; Japan SLC, Shizuoka, Japan) were maintained with standard diet Labdiet® 5L37 (Japan SLC) in a temperature-controlled room at 22 - 25°C with 12-h light/dark cycles, and acclimated for 1 week before animal experiments. Mice were fasted overnight but allowed free access to drinking water. Mice were then administered isosaponarin (50 mg/kg body weight) dissolved in distilled water by gavage. Twelve mice were housed in metabolic cages to collect the feces and sacrificed 8, 12, 24 and 48 h after the administration by collecting blood from the heart under anesthesia, while nine mice were housed in plastic cages and sacrificed 1, 2, and 4 h after the administration in the same way. Mice were physically normal without decrease in body weight and diarrhea throughout the experiment, and the food and water intake were not different from control mice. The mice were anesthetized with pentobarbital sodium, and the blood was collected from the heart with a heparinized syringe at corresponding time points. The stomach, small intestine, and large intestine were carefully removed, and the gastrointestinal remnants were separately collected by perfusion with 3 ml of ice-cold PBS and transferred to conical tubes. These tissues and remnants were immediately frozen by liquid nitrogen and stored at -80°C until HPLC analysis. The plasma was prepared from the blood by centrifugation at 450 × *g* for 15 min at 4°C. The 0-h control mice were administered nothing.

2.6 Extraction of Isosaponarin, Isovitexin and Apigenin from Plasma

Forty micro-liter of plasma was transferred into a microtube, and incubated at 37°C for 45 min with 40 µl of 0.1 M acetate buffer (pH 5.0) with or without 20 units of sulfatase/β-glucuronidase. The mixture was added 5 µl of 100 µM flavone as an internal standard and 450 µl of methanol, and agitated with a vortex mixer. After centrifugation at 2,000 × *g* for 15 sec, the 400 µl of supernatant was transferred to a new microtube. The residue was added 400 µl of methanol again and centrifuged at 2,000 × *g* for 15 sec to obtain methanol extract. This extraction process was repeated twice, and the sum of supernatant was evaporated with a centrifugal concentrator. The dried residue was dissolved in 100 µl of 50% (v/v) methanol and filtered through a 0.2-µm membrane filter, and analyzed on HPLC.

2.7 Extraction of Isosaponarin, Isovitexin and Apigenin from Gastrointestinal Tissues, its Remnants and Feces

The extraction of isosaponarin, isovitexin and apigenin from the tissues, gastrointestinal remnants and feces was performed according to the method described previously [21] with some modifications. In brief, the tissues; stomach, small intestine, and large intestine, were minced with surgical scissors, added 4 ml of 50% methanol (v/v) and 5 µl of 100 µM flavone as an internal standard, and homogenized thrice at 2,500 rpm for 7 sec by a cell disruptor, Multi-beads shocker. On the other hand, the gastrointestinal remnants and feces were homogenized with 6 volumes of 50% methanol (v/v) by the same method. The homogenates were agitated with a vortex mixer, sonicated for 15 min and centrifuged at 1,780 × *g* for 10 min. The supernatants were collected, agitated with a vortex mixer and filtered through a 0.2-µm membrane filter. The filtrate was subjected to a HPLC analysis.

3. RESULTS

3.1 Assembly of Caco-2 Intestinal Epithelial Model by 5-day Method

To investigate the intestinal absorption of isosaponarin, a Caco-2 intestinal epithelial model assembled by a 5-day method was used for isosaponarin permeability assay. The Caco-2 intestinal epithelial model was estimated by a TEER value and a lucifer yellow permeability assay. To compare a 5-day method used in the present study and a 21-day method used in the previous study [20], 3 nmol of quercetin or quercetin glucoside was subjected to the model assembled by a 5-day method in prior to the isosaponarin permeability assay. The amount of quercetin in the apical compartment was decreased in a time-dependent manner, while that of quercetin glucoside was hardly changed at 0.5, 1 and 2 h after the addition (Fig. 2). These results were consistent with the results of the previous report [20]. Therefore, the Caco-2 monolayer assembled by a 5-day method used in the present study was able to be used as an intestinal epithelial model for isosaponarin permeability assay as well as that by a 21-day method.

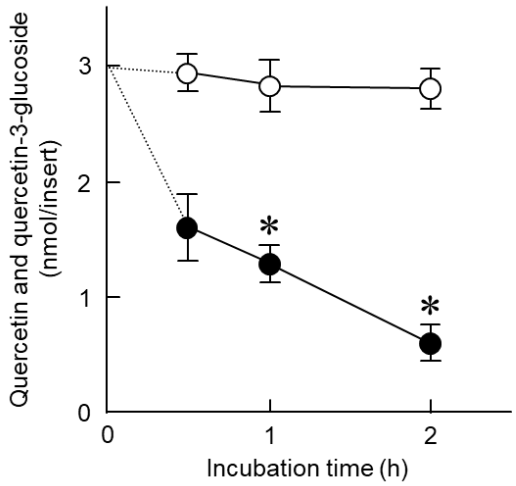


Fig. 2. Amounts of quercetin and quercetin glucoside in apical solutions

*Quercetin (solid circle) decreased rapidly after addition to the apical compartment, whereas quercetin glycosides (open circle) hardly decreased little after addition. Data are expressed as means ±SD (n=6). *p < 0.05 compared with the corresponding values at 0.5 h*

3.2 Isosaponarin Permeability Assay

Five hundred micro-liter of 10 μM isosaponarin (5 nmol) was added to the apical compartment of the Caco-2 intestinal epithelial model, and the amounts of isosaponarin and the prospected metabolites, isovitexin and apigenin, were measured by HPLC analysis in the apical solution, cellular extract and basolateral solution 0.5, 1 and 2 h after the addition (Table 1). Isosaponarin in the apical compartment slightly decreased to 4.9 nmol at 0.5 and 1 h, and 4.8 nmol at 2 h, while isosaponarin in the cellular extract and isovitexin in the apical compartment slightly increased. However, it is unlikely that these metabolism and absorption was due to the aggressive capacity of cells, because these changes were very small. Apigenin was undetected in any fractions. Thus, isosaponarin was hardly received metabolism and absorption in the Caco-2 intestinal epithelial model. In addition to isosaponarin, isovitexin was also subjected to the permeability assay with the Caco-2 intestinal epithelial model. More than 95% of isovitexin added to the apical compartment remained in the apical compartment 0.5 and 1 h after the addition. Apigenin were undetected in both apical and basolateral compartments (Table 2). These results indicated the isovitexin were also not received deglycosidation and absorption at the small intestine.

3.3 Oral Administration of Isosaponarin in Mice

To confirm the results from the Caco-2 intestinal epithelial model under the *in vivo* condition, mice were orally administered 2.5 μmol isosaponarin (approximately 50 mg/kg body weight) dissolved in distilled water, and the concentration of isosaponarin and the prospected metabolites, isovitexin and apigenin, in the plasma was determined by HPLC analysis. The recovery percentage of flavones, an internal standard, was more than 98.5%. The neither isosaponarin nor prospected metabolites were undetected in the intact plasma and sulfatase-treated plasma within 48 h under the HPLC condition used in the present study (Fig. 3). On the other hand, isovitexin was slightly detected in the homogenates of gastrointestinal tissues, *i.e.*, stomach (0.31% of equivalent amounts of isosaponarin administered mice), small intestine (4.9%), and large intestine (2.5%) (Table 3). Since these amounts were exceedingly small, isovitexin might be adsorbed on the surface of the tissues, *i.e.* isovitexin was not absorbed into the tissues. In addition, isosaponarin and apigenin were undetectable in these tissues (data not shown). These results indicate that

isosaponarin and/or the prospected metabolites are not absorbed into the body *in vivo*, and are consistent with the results from the Caco-2 intestinal epithelial model.

Table 1. The isosaponarin permeability assay with Caco-2 intestinal epithelial model

Incubation time (h)	Fractions	Isosaponarin (nmol/insert)[1]	Isovitexin (nmol/insert)	Apigenin (nmol/insert)
0.5	Apical solution	4.92 ± 0.06 (98.4 ± 1.2) [2]	0.083 ± 0.002 (1.65 ± 0.06) [3]	N.D.
	Cellular extract	0.13 ± 0.00 (2.55 ± 0.04)	N.D.	N.D.
	Basolateral solution	N.D.	N.D.	N.D.
1	Apical solution	4.88 ± 0.04 (97.6 ± 0.8)	0.085 ± 0.001 (1.70 ± 0.02)	N.D.
	Cellular extract	0.15 ± 0.01 (2.95 ± 0.01)	N.D.	N.D.
	Basolateral solution	N.D.	N.D.	N.D.
2	Apical solution	4.80 ± 0.07 (96.0 ± 1.4)	0.22 ± 0.02 (4.35 ± 0.40)	N.D.
	Cellular extract	0.17 ± 0.00 (2.95 ± 0.01)	N.D.	N.D.
	Basolateral solution	N.D.	N.D.	N.D.

Data are expressed as means±SD (n=6). N.D., not detected.
[1] The concentration of flavonoids without conjugation
[2] Parentheses show the recovery % of amounts of isosaponarin added to the insert (5 nmol/insert).
[3] Parentheses show the recovery % of equivalent amounts of isosaponarin added to the insert (5 nmol/insert).

Table 2. The isovitexin permeability assay with Caco-2 intestinal epithelial model

Incubation time (h)	Fractions	Isovitexin (nmol/insert)	Apigenin (nmol/insert)
0.5	Apical solution	4.77 ± 0.07 [1] (95.3 ± 1.3) [2]	N.D.
	Cellular extract	N.D.	N.D.
	Basolateral solution	N.D.	N.D.
1	Apical solution	4.87 ± 0.11 (97.3 ± 2.1)	N.D.
	Cellular extract	N.D.	N.D.
	Basolateral solution	N.D.	N.D.

Data are expressed as means ± SD (n=6). N.D., not detected
[1] Concentration of flavonoids without conjugation
[2] Parentheses show the recovery % of equivalent amounts of isosaponarin added to the insert (5 nmol/insert).

Table 3. Isovitexin in the gastrointestines of isosaponarin-administered mice

Time (h)	Stomach (μmol)	Small intestine (μmol)	Large intestine (μmol)
1	0.0078 ± 0.0025 [1] (0.31 ± 0.10) [2]	0.12 ± 0.05 (4.9 ± 1.9)	0.061 ± 0.031 (2.5 ± 1.2)
2	0.0023 ± 0.0015 (0.09 ± 0.06)	0.012 ± 0.003 (0.47 ± 0.12)	0.059 ± 0.003 (2.4 ± 0.1)
4	0.0023 ± 0.0018 (0.09 ± 0.07)	0.0045 ± 0.0035 (0.18 ± 0.14)	0.013 ± 0.003 (0.50 ± 0.12)
8	0.0025 ± 0.0018 (0.10 ± 0.07)	N.D.	N.D.

Data are expressed as means ± SD (n=3). N.D., not detected.
[1] Amounts of isovitexin without conjugation
[2] Parentheses show the recovery % of equivalent amounts of isosaponarin administered mice (2.5 μmol).

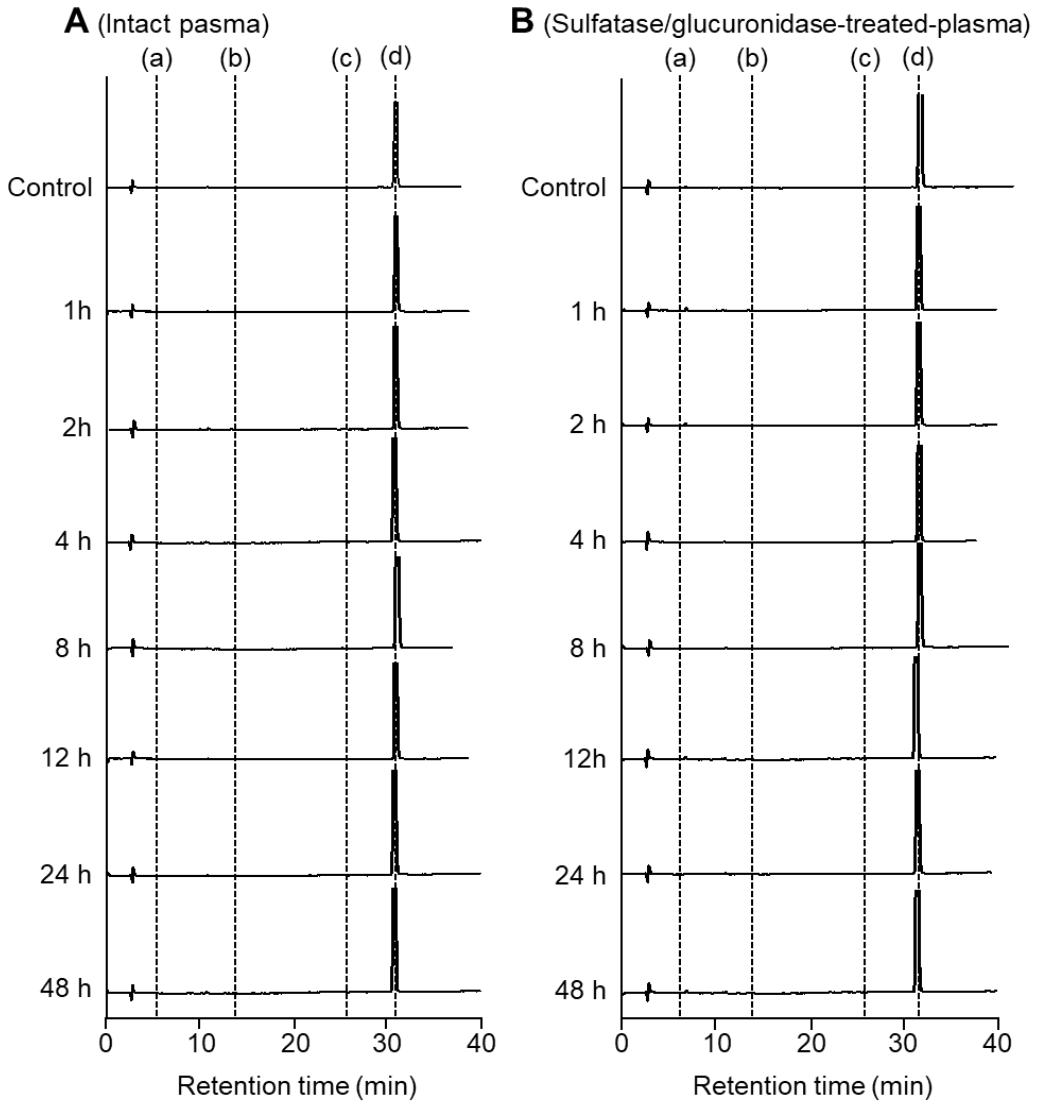


Fig. 3. Chromatogram of plasma after oral administration of isosaponarin

(a), (b), (c), and (d) indicated the retention times for isosaponarin (5.8 min), isovitexin (13.6 min), and apigenin (25.7 min), and (d) flavone (32.5 min) as an internal standard, respectively.

3.4 Isosaponarin and the Metabolites in the Gastrointestinal Remnants

Because isosaponarin and the prospected metabolites were unlikely to be absorbed into the body, the amounts in the gastrointestinal remnants and feces were determined by HPLC analysis. Thirty six percent of the original dose (2.5 μmol) remained in the gastric remnants 1 h after the administration, and isosaponarin gradually decreased to 1.7% at 12 h (Fig. 4A). Although isosaponarin was also detected in the small and large intestinal remnants, the maximum amounts were less than 1% of the original dose (Figs. 4B-C). Isovitexin equivalent to 8.6% of the administered isosaponarin was detected in the gastric remnants 1 h after the administration, and then the amount was gradually decreased to less than 1% at 12 h (Fig. 4A). The amounts of isovitexin in the small and large intestinal remnants were equivalents to 13% (Fig. 4B) and 28% (Fig. 4C), respectively, 1 h after the administration. The amounts of isovitexin in the small and large intestinal remnants were obviously higher than that of isosaponarin detected at the same time points.

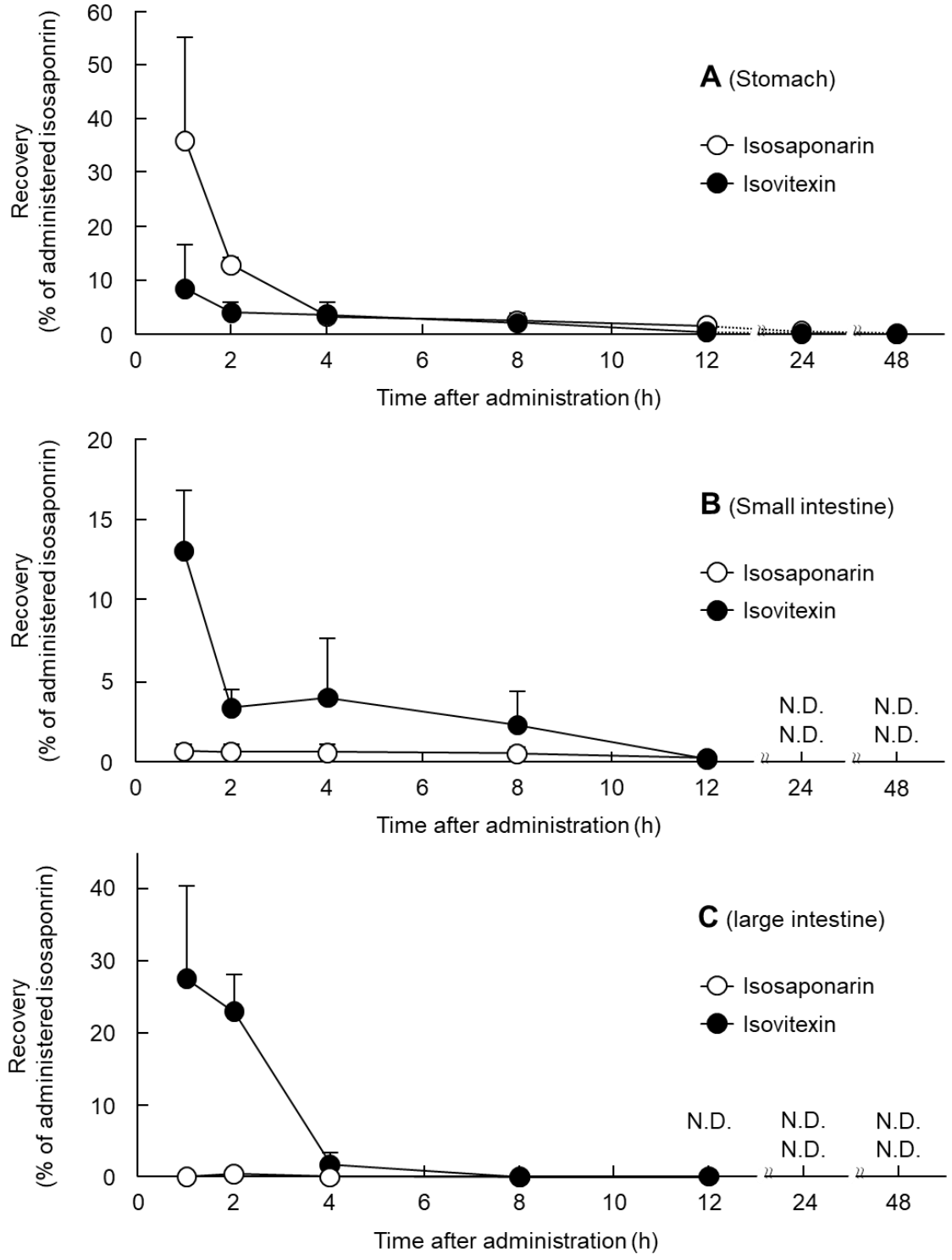


Fig. 4. The recovery of isosaponarin and isovitexin in the gastrointestinal remnants
Data are expressed as means ±SD (*n*=3). N.D., not detected.

Furthermore, isovitexin was detected in the feces but isosaponarin was not. The isovitexin excreted into the feces for 8 h accounted for 51% equivalents of dosed isosaponarin (Fig. 5), and did not increase thereafter. This result suggests that most of isovitexin was excreted into the feces within 8 h after the administration while a little isovitexin and isosaponarin remained in the

gastrointestinal remnants. Apigenin were not detected in the gastrointestinal remnants and feces throughout the experiments. These results indicated that isosaponarin was mainly metabolized to isovitexin in the stomach or small intestine, and most isovitexin was not absorbed into body and was excreted with feces.

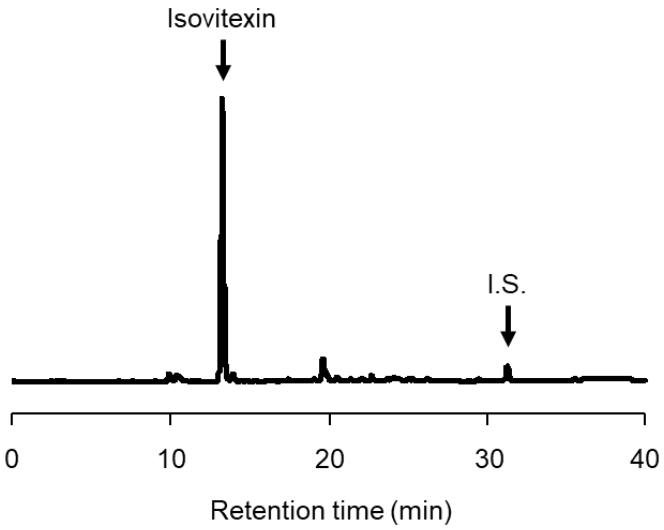


Fig. 5. Representative chromatogram of feces of mice

HPLC analysis detected isovitexin extracted from the feces, but not isosaponarin. Isovitexin and flavone as internal standard (I.S.) were detected at 13.6 min and 32.5 min, respectively

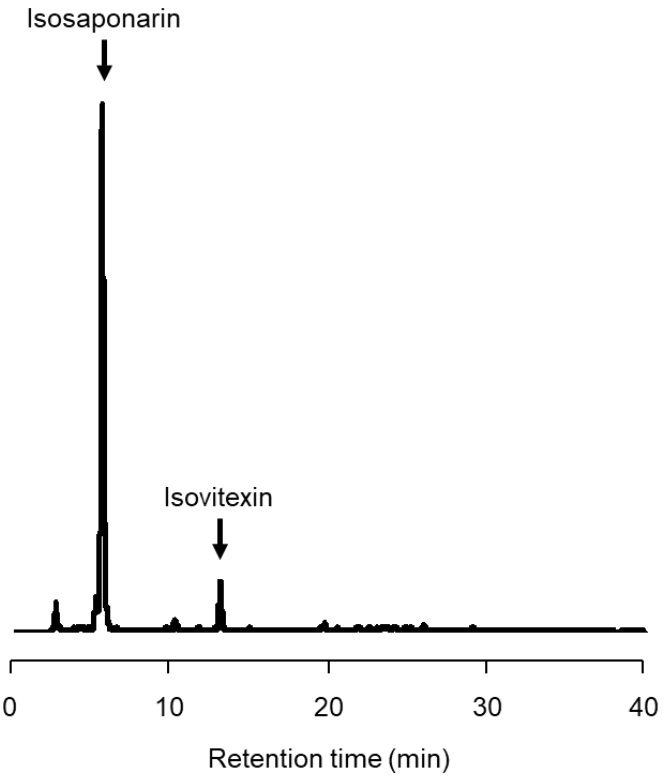


Fig. 6. Chromatogram of HCl-hydorolyzed isosaponarin

Isosaponarin and isovitexin were detected at 5.8 min and 13.6 min, respectively.

3.5 Hydrochloric Acid Hydrolysis of Isosaponarin *in vitro*

To investigate whether isosaponarin is metabolized to isovitexin by gastric acid in the stomach, 0.15 μmol of isosaponarin was incubated with 2N HCl at 37°C for 30 min. Isosaponarin very slightly decreased to 0.148 μmol, and isovitexin was slightly detected at 0.009 nmol (Fig. 6). Therefore, the acidic hydrolysis was minor deglycosidation of isosaponarin to isovitexin. This result suggests that isosaponarin is metabolized to isovitexin mainly in small intestinal tract, although a small amount of isosaponarin may be metabolized to isovitexin by gastric acid in stomach.

4. DISCUSSION

The aim of the present study was to investigate the absorption and metabolism of isosaponarin derived from wasabi (*Wasabia japonica*) leaves. The Caco-2 intestinal epithelial model and animal experiment indicated that isosaponarin and isovitexin were hardly absorbed into the body. The animal experiment suggested that isosaponarin was metabolized to isovitexin, and isovitexin equivalent to the approximately half of administered isosaponarin was excreted with feces within 8 h after the administration. Thus, the present study demonstrated that dietary isosaponarin was hardly absorbed into the body and preferably metabolized to isovitexin, and isovitexin was also hardly absorbed.

Isosaponarin (apigenin-6-*C*-glucosyl-4'-*O*-glucoside) have two glucose units in the structure; one is bound to the OH group at 4'-position on the B-ring of apigenin aglycone by *O*-glycosidic bond, and another one is bound to the carbon at 6-position on the A-ring by *C*-glycosidic bond. In the present study, apigenin was not detected in the any experiments; the Caco-2 intestinal epithelial model (Table 1), animal experiment (Fig. 3), and HCl hydrolysis experiment *in vitro* (Fig. 6), although isovitexin (apigenin-6-*C*-glucoside) was detected as an intestinal metabolite (Table 3, Figs. 4B-C). These results were indicated that deglycosylation of *O*-glucoside occurred in the gastrointestineal tract but the deglycosylation of *C*-glucoside was not. Quercetin-4'-*O*-glucoside was reported to be hydrolyzed by LPH [20], suggesting that *O*-glucoside at 4'-position on flavonoids is likely to be hydrolyzed by LPH. In the present study, the animal experiment demonstrated that isovitexin increased at small intestine immediately after the administration (Fig. 4B). Furthermore, the HCl hydrolysis experiment showed that most isosaponarin was hardly hydrolyzed by gastric acids though it was slightly influenced. These results suggested that isosaponarin was mainly metabolized to isovitexin by LPH, which hydrolyzed *O*-glucoside of isosaponarin at small intestine, while the acidic hydrolysis by gastric acid is also considered as the minor metabolic pathway of isosaponarin.

Zhang et al. [19] demonstrated that flavone *C*-glucosides; orientin (luteolin-8-*C*-glucoside), homoorientin (luteolin-6-*C*-glucoside), vitexin (apigenin-8-*C*-glucoside) and isovitexin were poorly absorbed in the gastrointestinal tract, and 21% of *C*-glucosides were excreted in the feces at 24 h. Thus, most flavonoid *C*-glycosides are unlikely to be absorbed at gastrointestinal tracts and excreted in the original form. In the present study (Fig. 3), isosaponarin and isovitexin were undetected in the plasma at any time points in this study, suggesting that isovitexin and isosaponarin was not absorbed in the original forms at the intestine. On the other hand, certain flavonoid *C*-glycosides were reported to be absorbed in the original form. For example, puerarin (daidzein-8-*C*-glucoside) was rapidly absorbed from the intestine without metabolism, and mainly excreted in the urine as the hydroxylated derivatives [22]. And it was also reported that puerarin was partially hydrolyzed to aglycone in the body [23]. Further study is needed to clarify the differences between the unabsorbed and absorbable flavonoid *C*-glycosides.

Flavonoid *C*-glycosides have been considered to contribute a diverse range of biological activities including the antimicrobial activity [24], and antioxidative activity [25]. Isosaponarin have been reported to promote the biosynthesis of type I collagen in human fibroblasts *in vitro* [6] and to inhibit to release glutamate in rat synaptosomes [7], but there is no information on the biological activity of dietary isosaponarin *in vivo*. Since dietary isosaponarin is immediately metabolized to isovitexin in the present study (Fig. 4), some health beneficial effects of isovitexin are expected rather than that of isosaponarin following the intake of isosaponarin. A recent study demonstrated that oral administration of isovitexin (15 mg/kg) has an anti-hyperglycemic action in rats [26]. Huang et al. [27] demonstrated that isovitexin suppressed the release of tumor necrosis factor α (TNF-α), production of prostaglandin E2, and expression

of cyclooxygenase-2 in lipopolysaccharide-activated RAW264.7 macrophages. It is well known that TNF-α production is increased under chronic hyperglycemia, and TNF-α has harmful effects on insulin sensitivity [28]. The suppressive effects of isovitexin on TNF-α production in intestinal macrophages [27] may contribute to the anti-diabetic activity *in vivo* [26]. In addition to isovitexin, daily oral administration of luteolin-6-*C*-glucoside (isoorientin) was reported to show subacute hypoglycaemic effect on streptozotocin-induced diabetic rats [29]. Thus, the consumption of flavonoid *C*-glycosides showed anti-diabetic activity *in vivo*. Dietary isosaponarin may also contribute to the prevention and treatment of diabetes.

Although most of isovitexin metabolized from isosaponarin were excreted with feces (Fig. 5), isovitexin might be also considered to be catabolized by intestinal microflora. Zhang et al. [19] proposed the metabolic pathway of isovitexin degradation initiated by the intestinal microflora; *i.e.*, intestinal microflora hydrolyzes *C*-glucoside of isovitexin and cleaves C-ring, resulting in the production of phloroglucinol and phloretic acid from A-ring and B-ring of apigenin, respectively. In this study, recovery amounts in the feces were approximately half of the administered isosaponarin (Fig. 5), indicating that the other half was considered to be catabolized to the small phenolic molecules such as phloroglucinol and phloretic acid by intestinal microflora. These phenolic compounds are reported to have a several biological activities; *e.g.*, the protective effects of phloroglucinol on ionizing radiation-induced cell damage through inhibition of oxidative stress *in vitro* and *in vivo* [30] the protective effect of phloroglucinol on myocardial ischaemia-reperfusion injury [31], and the antibacterial activity of phloretic acid [32]. It may be necessary to examine the pharmacokinetics of catabolites such as phloroglucinol and phloretic acid after ingestion of isosaponarin.

5. CONCLUSION

The present study showed that dietary isosaponarin is hardly absorbed into the body and metabolized to isovitexin in the gastrointestinal tract. In other words, the bioavailability of isosaponarin is very low, suggesting that they cannot be expected to have beneficial functions as dietary isosaponarin. On the other hand, most of isovitexin is excreted with feces, but isovitexin and its catabolites by intestinal microflora may be beneficial to human health. Further study is needed to elucidate the health-promoting effects of isovitexin and its catabolites.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chadwick CI, Lumpkin TA, Elberson LR. The botany, uses and production of *Wasabia japonica* (Miq.) (Cruciferae) Matsum Econ. Bot. 1993;47(2):113-35.
2. Hashimoto T, Yamada T, Nagai M, Yamada K, Tanaka M, Shimoaki T, et al. Wasabi. In: Govil JN,Singh VK, editors. Recent progress in medicinal plants ethonomedicune: Source & Mechanism, Studium Press LLC (USA). 2010;30:65-84.
3. Kinae N, Masuda H, Shin IS, Furugori M, Shimoi K. Functional properties of wasabi and horseradish. BioFactors. 2000;13:265-9.
4. Uto T, Fujii M, Hou D-X. 6-(Methylsulfinyl) hexyl isothiocyanate suppresses inducible nitric oxide synthase expression through the inhibition of Janus kinase 2-mediated JNK pathway in lipopolysaccharide-activated murine macrophages. Biochem Pharmacal. 2005;70:1211-21.
5. Morimitsu Y, Nakagawa Y, Hayashi K, Fujii H, Kumagai T, Nakamura Y, et al. A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. J Biol Chem. 2002;5:3456-63.
6. Nagai M, Akita K, Yamada K, Okunishi I. The effect of isosaponarin isolated from wasabi leaf on collagen synthesis in human fibroblasts and its underlying mechanism. J Nat Med. 2010;64(3):305-12.
7. Lu CW, Yeh KC, Chiu KM, Lee MY, Lin TY, Wang SJ. The effect of isosaponarin derived from wasabi leaves on glutamate release in rat synaptosomes and its underlying mechanism. Int J Mol Sci. 2022;23(15):8752.
8. Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans C. The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett. 1999;458(2):224-30.
9. Walgren RA, Lin J-T, Kinne RK-H, Walle T. Cellular uptake of dietary flavonoid quercetin 4'-β-glucoside by sodium-

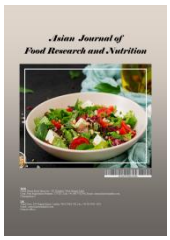
dependent glucose transporter SGLT1. J Pharmacol Exp Ther. 2000; 294(3):837-43.

10. Ioku K, Pongpiriyadacha Y, Konishi Y, Takei Y, Nakatani N, Terao J. β-Glucosidase activity in the rat small intestine toward quercetin monoglucosides. Biosci Biotechnol Biochem. 1998;62:1428-31.
11. Day AJ, Canada JF, Diaz JC, Kroon PA, Mclauchlan R, Faulds CB, et al. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett. 2000;468(2-3):166-70.
12. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. Free Radic Biol Med. 2004; 36,827-8.
13. Walle T. Absorption and metabolism of flavonoids. Free Radic Biol Med. 2004;36:829-37.
14. Graefe EU, Wittig J, Mueller S, Riethling AK, Uehleke B, Drewelow B, et al. Pharmacokinetics and bioavailability of quercetin glycosides in humans. J Clin Pharmacol. 2001;41:492-9.
15. Murota K, Shimizu S, Miyamoto S, Izumi T, Obata A, Kikuchi M, et al. Unique uptake and transport of isoflavone aglycones by human intestinal caco-2 cells: comparison of isoflavonoids and flavonoids. J Nutr. 2002;132(7):1956-61.
16. Abou-Zaid MM, Lombardo DA, Kite GC, Grayer RJ, Veitch NC. Acylated flavone C-glycosides from Cucumis sativus. Phytochemistry. 2001;58:167-72.
17. Krafczyk N, Glomb MA. Characterization of phenolic compounds in rooibos tea. J Agric Food Chem. 2008; 56,3368-76.
18. Joubert E. HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. Food Chem. 1996;55:403-11.
19. Zhang Y, Tie X, Bao B, Wu X, Zhang Y. Metabolism of flavone C-glucosides and p-coumaric acid from antioxidant of bamboo leaves (AOB) in rats. Br J Nutr. 2007;97:484-94.
20. Murota K, Shimizu S, Chujo H, Moon J-H, Terao J. Efficiency of absorption and metabolic conversion of quercetin and its glucosides in human intestinal cell line Caco-2. Arch Biochem Biophys. 2000;384(2):391-7.
21. Walton MC, Hendriks WH, Broomfield AM, McGhie TK. Viscous food matrix influences absorption and excretion but not metabolism of blackcurrant anthocyanins in rats. J Food Sci. 2009;74:22-9.
22. Prasain JK, Jones K, Brissie N, Moore R, Wyss JM., Barnes S. Identification of puerarin and its metabolites in rats by liquid chromatography-tandem mass spectrometry. J Agric Food Chem. 2004;52(12):3708-12.
23. Yasuda T, Kano Y, Saito K, Ohsawa K. Urinary and biliary metabolites of puerarin in rats. Biol Pharm Bull. 1995;18:300-3.
24. Hultin PG. Bioactive C-glycosides from bacterial secondary metabolism. Curr Top Med Chem. 2005;5:1299-331.
25. Fanz G, Grun M. Chemistry, occurrence and biosynthesis of C-glycosyl compounds in plants. Planta Med. 1983;47:131-40.
26. Folador P, Cazarolli LH, Gazola AC, Reginatto FH, Schenkel EP, Silva FR. Potential insulin secretagogue effects of isovitexin and swertisin isolated from *Wilbrandia ebracteata* roots in non-diabetic rats. Fitoterapia. 2010;81:1180-7.
27. Huang ST, Chen CT, Chieng KT, Huang SH, Chiang BH, Wang LF, et al. Inhibitory effects of a rice hull constituent on tumor necrosis factor α, prostaglandin E2, and cyclooxygenase-2 production in lipopolysaccharide-activated mouse macrophages. Ann N Y Acad Sci. 2005;1042:387-95.
28. Cao XY, Wang XH, Ma SL, Yang XJ, Wang XQ, Ding H, et al. Study of relationship between stress hyperglycemia and insulin-resistance related factors. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2006;12:751-4.
29. Sezik E, Aslan M, Yesilada E, Ito S. Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay- directed fractionation techniques. Life Sci. 2005;76(11):1223-38.
30. Kang KA, Zhang R, Chae S, Lee SJ, Kim J, Kim J, et al. Phloroglucinol (1,3,5-trihydroxybenzene) protects against ionizing radiation-induced cell damage through inhibition of oxidative stress in vitro and in vivo. Chem Biol Interact. 2010;185:215-26.
31. Li TT, Zhang YS, He L, Li NS, Peng J, Li YJ. Protective effect of phloroglucinol against myocardial ischaemia-reperfusion injury is related to inhibition of myeloperoxidase activity and inflammatory

cell infiltration. Clin Exp Pharmacol Physiol. 2011;38:27-33.

32. Huberman L, Gollop N, Mumcuoglu KY, Breuer E, Bhusare SR, Shai Y, et al. Antibacterial substances of low molecular weight isolated from the blowfly, *Lucilia sericata*. Med Vet Entomol. 2007;21:127-31.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97674



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 25-35, 2023; Article no.AJFRN.97627

Isolation and Characterization of Yeast Associated with Palm Wine Fermentation

Ejimofor Chiamaka Frances [a*], Nwakoby Nnamdi Enoch [b], Oledibe Odira Johnson [c], Afam-Ezeaku Chikaodili Eziamaka [c] and Mbaukwu Onyinye Ann [c]

[a] Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.
[b] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.
[c] Department of Botany, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97627

Original Research Article

Received: 14/01/2023
Accepted: 16/03/2023
Published: 03/04/2023

ABSTRACT

Wine is a naturally occurring beverage that is produced via the action of yeast cells from fruit juices. The purpose of this study is to produce orange fruit wine and evaluate its quality utilising yeast that has been isolated from palm wine. Although the yeast were isolated from old palm wine and characterised using conventional methods, Saccharomyces cerevisae was verified as the main species present. Palm wine was characterised to discover its physicochemical features. Using recipes that included a blend of each fruit must with Saccharomyces cerevisae isolated from palm

**Corresponding author: E-mail: cf.anyaegbu@coou.edu.ng, Chiamakanyaegbu@gmail.com;*

wine, orange fruit must was fermented for 14 days. Wine production was examined to assess its quality. For the yeast wine and commercial wine, respectively, the results indicate values of 3.67 and 3.38 for pH, 1.00 and 1.02 for specific gravity, 9.79 and 9.443 for percentage (%) alcohol (v/v), and 0.063 and 1.348 for percentage (%) titratable acidity. The results of the study shown that employing yeast (Saccharomyces cerevisae) isolated from palm wine, high-quality wine could be made from orange fruits for immediate consumption.

Keywords: Fermentation; isolation; palmwine; Upwine.

1. INTRODUCTION

The fermented sap of tropical plants of the palmae family is known as palm wine. It is both produced and consumed in enormous amounts in southeast Nigeria. It includes nutritionally significant elements such proteins, carbohydrates, vitamins, and amino acids [1]. These factors transform this wine into a true breeding ground for a variety of microorganisms, whose proliferation alters the physicochemical properties of the wine and fosters competition and successions of organisms.

Yeast is a single-celled, microscopic creature that belongs to the fungi family. Via the process of budding, in which a new cell starts as a tiny protrusion along the cell wall of a parent cell, individual yeast cells reproduce quickly. Huge numbers of yeast cells assemble in the presence of an ample food supply. Because of the brief two-hour budding period, the cells frequently resemble lengthy chains with freshly produced cells still linked to their parent cells [2].

Yeast are one of the few living things that can make energy without oxygen. Anaerobic refers to this absence of oxygen. Yeast breaks down starches and sugars into alcohol and carbon dioxide in these anaerobic conditions. Fermentation is the name given to this process. Enzymes, which function as catalysts in chemical processes and are akin to the digestive enzymes in the human body, are what cause yeast to ferment. The term "enzyme" really means "in yeast." The lengthy, chain-like molecules of starch are broken down into smaller sugar units by specific enzymes in yeast. Following that, different yeast enzymes change one type of sugar molecule into another [2].

The sugar molecule, which is made up of carbon, hydrogen, and oxygen atoms, is disassembled by further enzyme processes into ethyl alcohol and carbon dioxide. The chain of events gives yeast cells the energy they require for division and development (form of reproduction). In the natural world, yeast enzymes consume the sugar created by the breakdown of the intricate carbon compounds found in animal tissues and plant cell walls. Yeast serve as environmental natural decomposers in this way. Key Phrases Anaerobic: Residing or developing in an oxygen-deficient environment. Yeast is fed by the natural carbohydrates and sugars in the liquids. As a result of inadequate sugar breakdown when lacking oxygen during the fermentation process, yeast produces alcohol as a byproduct.

Indeed, several researchers have worked on projects to isolate and use palm wine yeasts in industrial operations. They include the generation of single cell proteins, portable ethanol production, and baking. *Saccharomyces cerevisiae* palm wine isolates were employed by Ogbonna [3] to make fake beer and fake palm wine, respectively. Characterizing these yeasts to produce fuel ethanol has not received much attention. Despite ongoing research into using bacteria to produce ethanol, yeast is still the preferred option for fermentation [4].

Saccharomyces cerevisiae yeast starters have been widely used in both commercial and homebrewing beverage manufacturing procedures since the 1980s. S. cerevisiae strains are currently used in the majority of wine production methods because they enable dependable and quick fermentations, lower the danger of slow or blocked fermentations, and guard against microbial contamination. In general, yeast starter cultures that are particularly chosen for the winemaking process on the basis of traits that have been scientifically validated complement and optimise the quality of the wine's distinctive qualities. In general, wines made using certain yeasts are of a higher calibre than those made by spontaneous fermentation [5].

The most well-known high-value fruit products are wine and fruit juice. The production of vinegar, a byproduct of making wine, may also employ it as a substrate. Although the methods

required in producing wine are rather simple, it can be difficult to create a commercial product (Amerine et al. 2020). Almost any fruit may be transformed into a wine that is excellent. Yeast that naturally exists in grapes may be used to ferment wine, albeit in nations where grapes aren't grown, other fruits are typically preferred for wine production. Wine is a product of alcoholic fermentation by yeast of ripe grapes or any fruit with a good proportion of sugar [1].

1.1 Statement of Problem

Researchers Anyaegbu et al. [4], Ejimofor et al. [5], Ejimofor and Oledibe [4], and many others have noted the presence of numerous microorganisms, particularly the bacteria and yeasts responsible for the fermentation of palm wine. The sugars in palm sap are converted during fermentation into alcohol and organic acids, which makes the sap less sweet. The sorts of bacteria that are present seem to be influenced by the sap's composition and fermentation stage [1]. Although yeasts frequently produce alcohol, bacteria seldom do so (Ingraham and Ingraham, 2014). For the majority of alcoholic drinks, yeast is employed. Pulque, however, is an exception. The alcoholic beverage pulque is made from the agave plant's juice and *Zymomonas mobilis* [6]. Due to the ability of likely Zymomonas species and other microorganisms present in the wine to ferment, it is difficult to store palm wine and maintain its normal characteristics. This has been a significant issue in the bottling of palm wine in Nigeria and subsequently its distribution for consumption. So, the existence of yeast species in palm wine may be advantageous to man in addition to their capacity for fermentation.

So, the goal of the current study is to isolate and identify the yeast in palm wine and to ascertain how the bacteria contributes to the fermentation of carbohydrates during wine production.

The major aim of this project work is to identify and isolate yeast commonly found in palm wine and evaluates their role in production of wine.

The specific objectives are:

Collection of palm wine samples from different sites within Awka.

- Determining and separating the microbes in palm wine.
- Using the yeast isolate to make wine
- Making a comparison between commercial yeast and isolated yeast.
- Gathering data on the results, comparing it to the literature that is accessible, and providing the required suggestions.

1.2 Significance of Study

The microorganisms found in palm wine have significant economic value and have helped both people and enterprises. The findings of this research will be significant in the following ways:

- The research will provide additional information about the nutritional value of palm wine and the need to boost consumption. The sales revenue for producers and marketers of palm wine will rise as a result.
- This effort will demonstrate the value of yeast in the creation of wine.
- The outcome of this investigation would be beneficial to the beverage industries since it will suggest several locations where the detected microorganisms may be used as a catalyst in the industries. The research will also present new techniques for fermentations that use less dangerous microbes. This action will gain more for such industries.
- Researchers that wish to pursue the isolation of the numerous bacteria discovered via this effort will have a solid basis thanks to this work. These bacteria can be utilised for biotechnology in the alcoholic beverage industry. Lastly, the government and our community will gain from this effort in the form of new businesses being started by individuals who have learned about the fantastic nutritional value of this palm wine.

2. MATERIALS AND METHODS

2.1 Sample Collection

From tappers in Awka, five samples from each of the two sources of palm wine (Raffia palm and palm tree) were chosen at random. The palm wine was gathered by the tappers utilising natural wood during tapping process using bamboo tube. Following that, the palm wine was collected in sterile bottles and transported (30 minutes) to the laboratory while being preserved at 40°C in the icebox.

2.2 Preparation of Samples

Within a day, the physical and chemical characteristics of each sample of palm wine were established. The samples were sterilised using Watman filter paper and aseptically filtered before being stored at 40°C for analysis.

2.3 Physicochemical Properties

Visual inspection of the samples of palm wine was required for this.

A hunter lab clorflex colorimeter was used to measure the samples' colour.

- According to Taipaiboon's instructions, the transmittance at 650 nm was measured using a spectrophotometer to assess the turbidity of the palm wine (13).
- The palm wine's flavour and aroma were also assessed.
- A pH metre calibrated with pH 4.0 and 7.0 was used to measure the pH value at room temperature.
- Using phenolphytalin as an indicator and calculating the total acidity in terms of lactic acid, the total acidity was evaluated by titration with NaOH.
- Using a hand refractometer, the total soluble solids in the palm wine sugar syrup were calculated as a degree Brix.
- By titrating with Fehling reagents, total sugar and reducing sugar concentrations were determined. Grams of glucose per 100 grammes of sample were used to express the results.
- An Orion 4 Stars conductometer was used to assess conductivity. The process involved calibrating the device with standards of 1413 S and 12.9 mS/cm, then submerging the sensor in tequila and measuring the conductivity in triplicate. Each time the electrode was submerged, it was thoroughly cleaned with water. At room temperature, all tests were carried out.
- An Anton Paar DSA5000 densimeter and sound velocity analyzer with a new-generation stainless-steel cell was used to measure density and ultrasonic velocity.
- Using a Peltier element that had a precision of 0.001°C for temperature control, errors in density of around g/cm3 resulted. The resolution in this investigation was 102 m/s, and temperature fluctuations are the primary source of errors in ultrasonic velocity measurements. After the prescribed procedure, which involved repeatedly injecting Alconox at a 40% concentration, the densimeter was cleaned. Following that, ultrapure water was pumped to calibrate at 20°C until density was measured at 0.998203 g cm3. When this measurement was made, the samples of tequila's density and viscosity were determined. At 25°C, duplicate measurements of the tequilas' density and sound velocity were made. The measurements of density and sound velocity were done simultaneously.
- A refractometer made by the manufacturer Abbe, model 2WA, was used to measure the refractive index of the tequila. Ethylic alcohol was used to first clean the prism before being calibrated with a drop of pure ethylic alcohol. The equipment's viewing field was modified to light up half of the area while leaving the other half in the dark. When everything was in working order, a measurement of 1.36 was made. Tequila was afterwards dropped into the sample holder to measure its refractive index. At 25°C, each measurement was made three times.
- An ARES rheometer TA-22 G2 was used to evaluate the viscosity of several tequilas utilising double-wall Couette geometry. The cup's inner and outer diameters are 27.94 and 34 mm, respectively, while the hollow cylinder's inner and outer diameters are 29.51 and 32 mm, respectively. At 25 °C, all measurements were made. The sample container was filled with 8 mL of tequila, which was kept moving at a shear rate of $10s^{-1}$.

2.4 Isolation of Yeasts from Palm Wine

The 25-day-old wine samples were centrifuged for five minutes at a low speed in sterile centrifuge bottles. By streaking on Glucose Yeast Agar plates, one ml of the serially diluted sediment is inoculated and incubated at 28°C for 24 hours [4]. By additional streaking on GYA, the yeast colonies that formed are separated and purified. According to Kunkee and Amerine [7], physical traits and patterns of fermentation were used to identify yeast isolates.

2.5 Yeast Identification

Ejimofor et al. [8] identification keys and common morphological and physiological tests were used for the isolation and identification of yeasts. Incubation took place at 28°C under aerobic circumstances. After isolation on glucose yeast agar (GYA) and yeast malt agar (YMA), the morphological and cultural traits of the years were examined (Biolife). These examinations covered morphology, surface traits, the development of ascospores, the presence of pseudohyphae, and vegetative reproduction. Sugars such glucose, galactose, sucrose, maltose, cellobiose, trehalose, lactose, raffinose, soluble starch, D-xylose, L-arabinose, and Dribose were tested for their ability to ferment. Another experiments include nitrate absorption, growth in 10% NaCl + 50% glucose in yeast extract, growth at 37°C and growth in 50% w/w glucose yeast extract.

2.6 Evaluation of Yeast Strains Isolated with Commercially Sold Yeast

The yeast isolated was used for the production of wine and compared with commercially sold yeast.

2.7 Sample Preparation

Purchased fresh oranges were separated, properly cleaned with clean water to eliminate any clinging materials, peeled, and had their seeds removed. With a sharp stainless steel knife, the flesh's (3.3 kg) little pieces were divided into pure juice. The fluid was filtered through a mesh cloth to eliminate any remaining solids. After being extracted, the juice was put into clean glass bottles and pasteurised for 30 minutes at 70°C using a heating mantle. In addition to 10g of aspertine, 10g of citric acid was added.

2.8 Grape Wine Fermentation

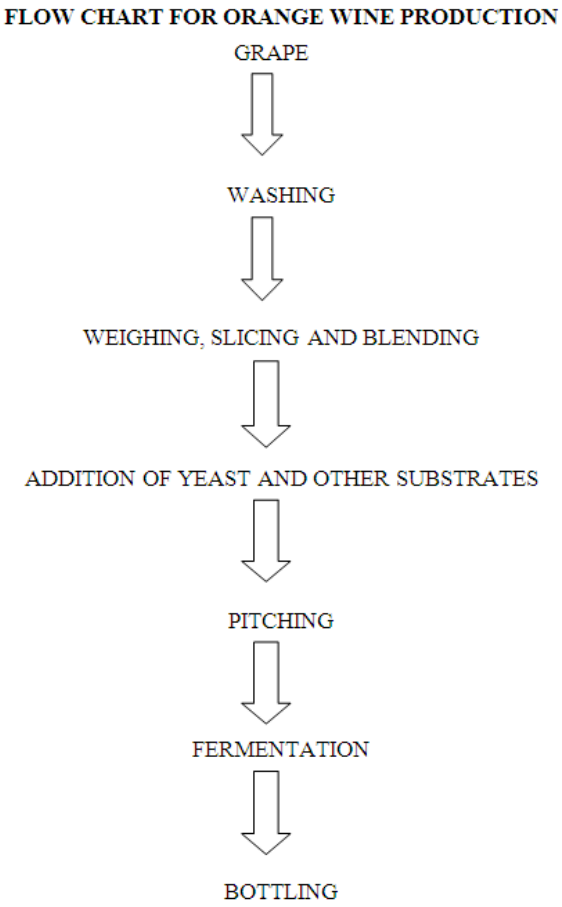


Fig. 1. Flow chart for grape wine production

2.9 Statistical Test

Yeast count, total suspended particles, total dissolved solids, titrable acidity, pH estimation, specific gravity, and alcohol concentration were all sampled every 48 hours.

3. RESULTS

The physicochemical properties of the palm wine and up wine used in the production of wine are presented in Table 1.

Table 1. Physicochemical properties of palm wine and up wine

Parameter	Palm wine	Up wine
Colour	Milky	Cloudy
Alcohol Content (g/100ml)	4.0	4.3
Density	1.02	1.03
pH	7.20	6.0
Glucose (g/100ml)	0.60	0.75
Fructose (g/100ml)	0.80	1.05
Sucrose (g/100ml)	2.50	2.90
maltose (g/100ml)	0.09	1.80
Total Sugar (mg/100ml)	3.99	6.50

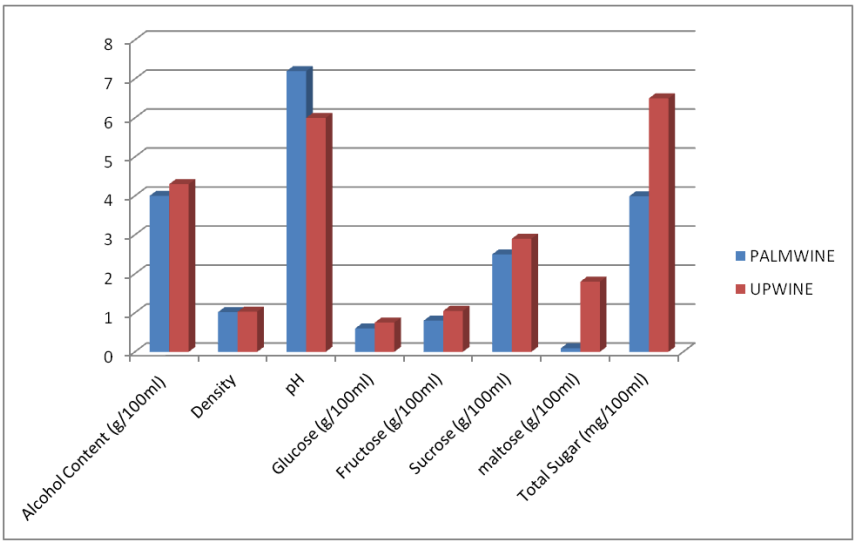


Fig. 2. Physicochemical properties of palm wine and up wine

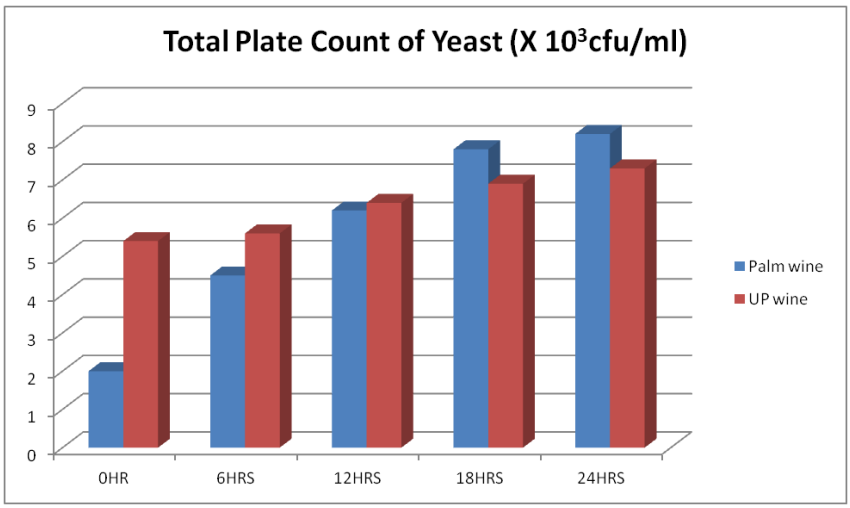


Fig. 3. Total Plate Count of Yeast (X 10^3cfu/ml) carried out at different times

Table 2. Total Plate Count of Yeast (X 10^3cfu/ml) carried out at different times

Time (Hrs)	0	6.0	12.0	18.0	24.0
Palm wine	2.0 X 10^3	4.5 X 10^3	6.2 X 10^3	7.8 X 10^3	8.2 X 10^3
UP wine	5.4 X 10^3	5.6 X 10^3	6.4 X 10^3	6.9 X 10^3	7.3 X 10^3

Morphological Characteristics of yeast cells

Isolates	Surface	Margin	Colony Size (mm)	Shape	Vegetative Reproduction	Probable Isolates
A	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
B	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
C	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
D	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
E	Smooth	Entire	0.5 cream	Spherical	Budding	*S. globosus*
F	Smooth	Entire	0.3 cream	Elipsoidal	Budding	*S. cerevisiae*

Carbohydrates Fermentation by Yeast Isolates

Carbon Source	A	B	C	D	E	f
Glucose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Lactose	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Xylose	-	-	-	-	-	-
Raffinose	+	+	+	+	+	+

Yeast isolates, sources, Name, sedimentation rate and ethanol tolerance

Isolate	Source	Name	Sedimentation rate	Ethanol tolerance
A	PALM WINE	*S. cerevisiae*	57.5	12.0
B	PALM WINE	*S. cerevisiae*	56.5	10.0
C	PALM WINE	*S. cerevisiae*	83.6	12.0
D	PALM WINE	*S. cerevisiae*	82.0	17.0
E	UP WINE	*S. cerevisiae*	90.0	16.0
F	UP WINE	*S. globosus*	64.5	15.0

Table 3. Physiochemical properties of yeast wine and commercial wine

Parameters	Yeast wine	Commercial wine
pH	3.67	3.38
Specific gravity	1.00	1.02
Titratable acidity	0.63	1.34
Residual ºBx	0.54	0.54
Alcoholic content percentage (%) (v/v)	9.46	9.44

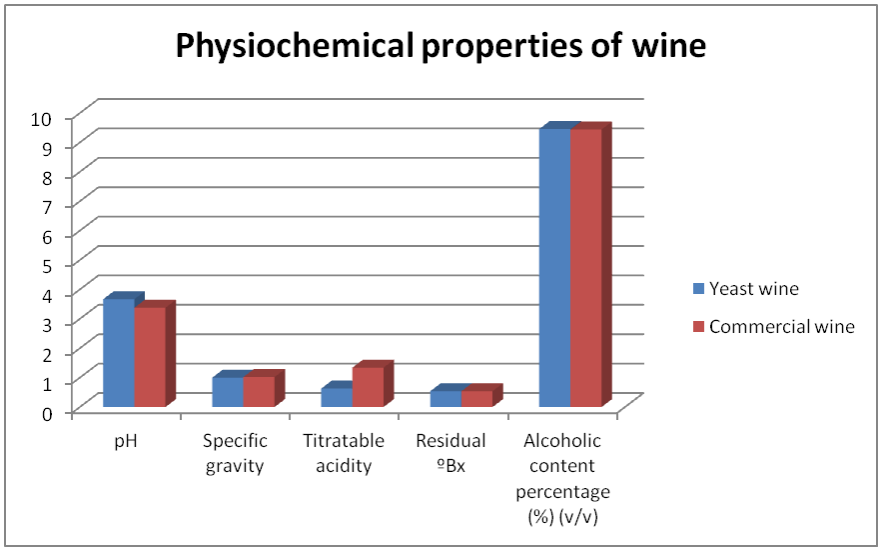


Fig. 4. Physiochemical properties of yeast wine and commercial wine

Table 4. Summary of the mean sensory score for the yeast wine and commercial wine

Parameter	Yeast wine	Commercial wine
Colour	6.80± 0.11	7.50± 0.25
Odour	7.50± 1.03	7.40± 0.20
Taste	7.30± 1.20	6.70± 0.11
Overall acceptability	7.20± 0.33	7.90± 0.20

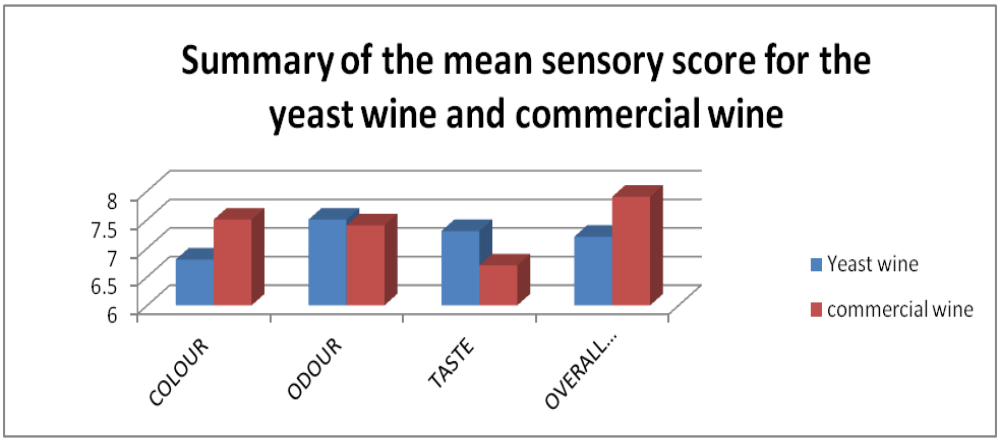


Fig. 5. Summary of the mean sensory for the yeast wine and commercial wine

3.1 Microbial Count

The Total Plate Count of Yeast (X 10^3cfu/ml) carried out at different times viz; 0, 6, 12, 18 and 24 hours respectively are presented in Table 2.

3.2 Physiochemical Properties of Wine

The wine produced after 14 days fermentation with yeast isolated from palmwine and commercial yeast were compared in other to evaluate its quality. The result shows values of 3.67 and 3.38 for pH, 1.00 and 1.02 for specific gravity, 9.64 and 9.44 for percentage (%) alcohol (v/v), and 0.63 and 1.34 for percentage (%) titratable acidity respectively for the yeast wine and commercialwine. The physiochemical properties of the yeast wine and commercialwinene are presented in Table 3.

3.3 Sensory Properties

The sensory evaluation result of yeast wine and commercial wine are presented in in Table 4. The result revealed that wine fermented with yeast differed significantly in terms of color, odour, taste, and overall acceptability when compared with the control sample.

4. DISCUSSION

Using yeast that was isolated from palm wine, we investigated the fermentation process used to make wine. Fruit that had been crushed was used in every step of the procedure. Some of the orange experimentation also used the fresh pulp that had been squeezed. The orange fruit mash had an initial sugar content of 110.1 g/L. While the lag phase was shorter and the fermentation rate was comparable, the yeast-inoculated alcoholic fermentation occurred more quickly than the spontaneous one. Although particular yeast for inoculation will be highly recommended in the industrialization of both the wine and vinegar processes, yeast inoculation was not actually necessary to generate these fruit wines. Shorter production cycles and a repeatable product are required by industrialization, and these requirements might be met by the process of inoculating certain strains [8].

The ultimate product yield (wine) is satisfactory because it was consistently well above 60%. We carried out the entire procedure in the lab, with such constraints as the press's force and the small-scale recovery of fruit pulp. Higher yields will result by scaling up to larger amounts and using industrial machinery, similar to what is seen in wine. In both instances, the finished product had pleasing colour and organoleptic qualities.

According to Table 5, the yeast wine had a lower acidity than commercial wine. This rise in acidity is likely caused by a few organic acids that are present in yeast wine and are mostly used as preservatives. According to Ough [9], main acids produced during fermentation include lactic, malic, succinic, and acetic acids. At a final acidity of 0.63%, spoiling organisms can be prevented. As reported by Pozo-Bayón et al. [10], table wines have titratable acidity in the range of 0.6-0.9%. These acids must be present in wine for it to function properly; otherwise, the beverage would taste unpleasant and deteriorate with a bad colour and flavor [11].

In line with expectations, the pH dropped from 3.67 in sycamore wine to 3.38 in yeast wine. This ought to follow from its inverse relationship to acidity. Yet it's crucial to note that there is no direct correlation between pH and total titratable acidity due to the fermenting liquor's variable buffer capacity [12]. As a result, the fact that yeast created few acids throughout fermentation accounts for the low change in fixed acidity [3]. For the microbiological stability of sycamore wine aroma and flavour developments, this PH value of 3.67 is crucial.

While fermentation continues, the alcohol content gradually rises. From the 14th day, which was the final day of fermentation, it thereafter became steady. The majority of the fermentable carbohydrates have been transformed to alcohol, which is the cause. Also, the toxicity of the created additional alcohol rendered the yeast dormant for further synthesis [13]. Its alcohol concentration was 9.67%, whereas that of commercial wine was 9.44%, mostly as a result of the further conversion of residual sugars to alcohol. Our findings are consistent with table wines' typical alcohol concentration, which runs from 6 to 10% [14].

The yeast wine's specific gravity was 1.00 as opposed to commercial wine's 1.02, but the difference was due to the conversion of sugar to alcohols, which has a lower specific gravity than sugar. This outcome supports the findings of Akubor et al. [15], who created wine using sycamore and bush mango juice, respectively.

The asessors gave the wine good marks for its sensory quality features and confirmed this by saying they would buy the wine if it were put up for sale. The assessors stated that this wine had a fruity-like flavour with a noticeable orange taste. According to Olorunfemi et al. [16]'s research, yeast, ambient conditions, and physiochemical processes all have a role in the kind and scent of wine that was created.

The resulting orange wine is clear and brown in hue. This was brought on by the orange pulp must's brown tint and prolonged age time. Moreover, it was seen that particle sedimentation happened quite quickly. This may have happened because they included denser, insoluble particles, which sink to the bottom. The papain protease enzyme's activity guaranteed that the proteins, peptides, and polypeptides present in the wine were broken down. After the wine was allowed to stand for a while, the

protein-tannin complex, which also included the peptide and polypeptide components, settled out. This improved wine's ability to clarify as it aged. The wine's peptide and polypeptide components formed a complex with the tannis (protein tannin complex), which settled out throughout one month of maturing, resulting in the mean value of colour acceptability derived from the sensory assessment being 6.80 and 7.50 for the standard [15].

For the odour of orange wine, sensory assessment mean scores were 7.4 for commercial wine and 7.5 for yeast wine. This demonstrated that there is no discernible difference between the yeast wine and the control wine in terms of aroma. There is no discernible difference between the commercial wine and yeast wine in terms of taste, according to Table 1, which is consistent with Mounigan et al. [17] observations in sensory acceptance, quantitative descriptive, and physicochemical examination of wines. For the standard, this was done with 7.2 and 7.9. This demonstrated that there are no appreciable differences.

5. CONCLUSION

This investigation proved that many Saccharomyces species were isolated from old palm wine and upwine. The ability of the isolate to develop on 10% sodium chloride + 50% glucose medium revealed that the isolate is *Saccharomyces cerevisae*. The isolated species displayed significant similarities. *Saccharomyces cerevisae* may be distinguished from all other species by the growth test. The potential exists that yeast isolated from palm wine might be used to make orange wine. As compared to a typical egg wine sample, the wines generated did not significantly differ in terms of pH, specific gravity, percentage (%) alcohol (v/v), or percentage (%) titratable acidity. The outcome of this research has demonstrated that the process of manufacturing orange wine, which began with the harvesting of healthy sycamore and garden egg fruit, washing, crushing, adding sulphites, fermenting, racking, clarifying, packing and pasteurization and finally aging of the wine can be achieved successfully. The wine made held up well against a table wine purchased from the market, which prompted measures to fully halt the importation of fruit wines. The capability of its production on an industrial scale is confirmed by the successful manufacture of orange wine from palm wine yeast. It will go a long way towards resolving the issue of all fruits being wasted in the nation, particularly seasonal fruits, and by doing so waste management will be improved. So, it is feasible to produce high-quality, delectable, and acceptable wine from sycamore pulp.

6. RECOMMENDATIONS

The effectiveness of locally isolated yeast (*Saccharomyces cerevisiae*) from palm wine for the creation of fruit wine serve as the foundation for this study. The findings of the fermentation indicate that palm wine must might be used to make respectable wine. During the alcoholic fermentation as suitable and acceptable substrates for wine production, the study also shed light on the effectiveness and function of local yeast strains. This study has shown that it is feasible to create wines with high acceptance and appropriate microbiological standards from fruit that is readily available locally.

In this investigation, yeast from palm wine was isolated and identified. It was demonstrated that the enrichment culture approach is effective at encouraging yeast growth. It was advised that these fruits be fully ripe in order to be used for the enrichment procedure. The isolates generated the maximum acetic acid content and could grow at ethanol concentrations of 4–10%, indicating their viability for making vinegar. To determine the wines' shelf life, however, more investigation is required.

COMPETING INTERESTS

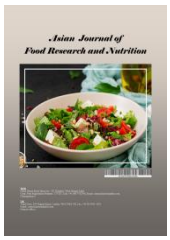
Authors have declared that no competing interests exist.

REFERENCES

1. Okafor N. Microbiology and biochemistry of oil-palmwine. Advances in Applied Microbiology. 2017;24:237-256.
2. Ingram T, Burttke KC. The leavening activity of yeasts associate with palm wine. J. Appl Bacterio. 2014;64;235-240.
3. Ogbonna A. Isolation of yeast from raffia wine. Journal of Applied Microbiology. 2014;113(6):1428-144.
4. Anyaegbu CF, Oledibe OJ, Amadi JE. Effect of bakers yeast (*Saccharomyces cerevisiae*) in the production of wine using oranges, apples and pineapples. European Journal of Biology. 2019;4 (3):41-55.
5. Ejimofor CF, Oleidibe OJ. Production of wine from red muscat grapes using

brewers yeast (*Saccharomyces cerevisiae*). Nigerian Journal of Mycology. 2021;13:51-63.
6. Uzochukwu SV, Balogh E, Tucknott OG, Lewis MJ, Ngoddy PO. Role of palmwine yeast and bacteria in palmwine aroma. Journal of Food Science and Technology. 2019;36(4):301-304.
7. Kunkee H, Amerine CE. Production of red wine from roselle (Hibiscuss abdariffa) and pawpaw (Caricapapaya) using palm wine yeast (*Saccharomyces cerevisiae*). Nigerian Food J. 2010;25(2):158-164.
8. Ejimofor, Chiamaka Frances, Oledibe Odira Johnson and Mendu Ebere Frances. Isolation and identification of microorganisms in wine produced from red muscat grapes. Asian Journal of Plant and Soil Sciences. 2021;6(3):21-27.
9. Ough RC. Use of high ethanol resistant yeast isolates from Nigerian Palm wine in larger beer brewing. World J. Micro. Biotech. 2010;9(6):660-661.
10. Pozo-Bayón RJ, Rodríguez-Álvarez JA, Valenzuela-Encinas FA, Gutiérrez-Miceli FA, Dendooven L. The bacterial community in “taberna” a traditional beverage of Southern Mexico. Letters in Applied Microbiology. 2012;51(5):558-563.
11. Reddy WK, Sampson E, Tano-Debrah K. Growth of yeasts, lactic and acetic acid bacteria in palmwine during tapping and fermentation from felled oilpalm. Elaeis guineensis in Ghana. Journal of Applied Microbiology. 2015;102(2):599–606.
12. Lea FN, Durán-Quintana MC, Ruíz-Barba JL, Querol A, Garrido Fernández A. Use of molecular methods for the identification of yeast associated with table olives. Food Microbiology. 2013;23(8):791-796.
13. Gambell, Santaroni MJ. Production of coyol wine from *Acrocomia mexicana* (arecaceae) In Honduras. Economic Botany. 2014;44(1):84-93.
14. Ferreira G, Jimenez V, Talaro T. Commercial fruit and vegetable products. Microbiology and Biotechnology. 2015; 2:681-707.
15. Akubor, Abalaka G Okpara BN. Characterization of palm wine yeast isolates for industrial utilization .African J. Biotechnol. 2013;5(19):1725-1728.
16. Olorunfemi B, Belloch C, Uruburu F, Querol A. Identification of yeasts by RFLP analysis of the rRNA gene and the two ribosomal internal transcribed spacers. International Journal of Systematic Bacteriology. 2019;49(1):329-337.
17. Mounigan N, Pina C, Mendes F, Couto JA, Hogg T, Vasconcelos I. Volatile compounds contribution of Hansenia sporaguilliermondii and Hansenia sporauvarum during red wine vinifications. Food Control. 2016;22(5):662-667.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97627



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 36-47, 2023; Article no.AJFRN.97812

Proximate, Mineral and Microbial Analysis of Locally Produced Juice (Kunu, Soymilk and Tigernut)

Ejimofor Chiamaka Frances [a*], Nwakoby Nnamdi Enoch [b], Oledibe Odira Johnson [c], Afam-Ezeaku Chikaodili Eziamaka [c] and Mbaukwu Onyinye Ann [c]

[a] Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria.
[b] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria.
[c] Department of Botany, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97812

Original Research Article

Received: 18/01/2023
Accepted: 20/03/2023
Published: 10/04/2023

ABSTRACT

Kunu, soymilk and tiger nut drinks are locally produced indigenous non-alcoholic beverages widely consumed in Nigeria. The beverages sold in Akwa was analysed for proximate, mineral and microbial analysis. The AOAC method of analysis was employed in the determination of proximate and mineral composition of the drinks. The following proximate results were obtained, soymilk contained 86% moisture, 2.22% ash, 0.07% fiber, 4.40% protein, 1.37% fat and 5.94% carbohydrate. For kunu; 81% moisture, 1.85% ash, 0.53% fiber, 1.85% protein, 0.81% fat and

**Corresponding author: E-mail: cf.anyaegbu@coou.edu.ng, chiamakanyaegbu@gmail.com;*

13.96% carbohydrate. For tiger nut; 84%moisture, 3.08% ash, 0.14% fiber, 2.70% protein, 1.95% fat and 11.03% carbohydrate. Mineral analysis of soy milk contained 127.89 Ca, 0.85 Fe, 17.60 P, 147.00 Mg and 11.37 P. Kunu contained 217.90 Ca, 2.37 Fe, 113.00 K, 106.05 Mg and 20.00 P. Tiger nut contained 221.00 Ca, 2.83 Fe, 135.00 K, 175.00 Mg and 75.10 P. Total bacteria count of soymilk, kunu-zaki and tigernut ranges from (0.70×10^6 to 1.97×10^6) (cfu/ml), (0.60×10^6 to 1.90×10^6) (cfu/ml), (0.40×10^6 to1.59×10^6) (cfu/ml) respectively. Faecal bacterial count of soymilk, kunu-zaki and tiger nut ranges from (3.00×10^4 to 5.90×10^4) (cfu/ml), (3.37×10^4 to 5.50×10^4) (cfu/ml), (1.15×10^4 to 5.13×10^4) (cfu/ml). Bacteria identify are *Klesiellaspp*, *Salmonella spp*, *Shigellaspp*, *E. coli*, *Vibrio spp*, *Staphylococcus aureus* and *Pseudomonas spp.*

Keywords: Bacteria; Drinks; Kunu; soymilk; tigernut.

1. INTRODUCTION

Kunun-zaki (Kunu) is a cereal based non-alcoholic fermented beverage mostly consumed in the Northern part of Nigeria. It can be produced either from millet (*Pennisetumtypoidum*), Sorghum (*Sorghum bicolor*), or maize (*Zea mays)* Akoma et al. [1]. Kunun-zaki is a Hausa word meaning sweet beverage. It is consumed anytime of the day by both adults and children as a breakfast food drink. It is a refreshing drink usually used to entertain visitors; it also serves as an appetizer and is commonly served at social gathering [2]. Onuorah et al. (1987) reported kunun-zaki as being regarded as after meal drinks or refreshing drinks in rural and urban centres, it is sometimes used as a weaning drink for infants [3]. Preparation methods vary amongst people's taste and cultural preferences. Production of kunun-zaki is still on small scale and the beverage is widely found in the local market and at resorts [4]. This non-alcoholic beverage is however becoming more widely accepted in several other parts of Nigeria, owing to its refreshing qualities [5].

1.1 Soymilk

Soymilk gotten from soybean (*Glycine max*) is a member of the family *legminosae* sub family *papilnonaceae* which have an exceptional nutritional and functional food profile. Soy-foods are considered to be nutritious and healthy based on their nutrient composition [6]. It is an excellent source of protein and oil of good quality. It contains about (43%) protein, (21%) carbohydrates, (5%) minerals, (8%) moisture, (20%) fat, (4%) fiber [7]. Soybean is rich in calcium and vitamin B12. Tocopherols are an important constituent of soy oil, due both to the vitamin E supplied for human nutrition and their antioxidant properties.

Soy bean was introduced into Nigeria in 1908; it was first planted in Ibadan, Oyo State. Initially the crop was cultivated for export with the support and encouragement of Groundnut Board. Nigeria presently produces about 500,000 MT of Soybean annually making it the largest producer of the product on the African continent. As this drink is cholesterol free and low in energy, it could enhance health benefits in terms of reducing body weight and blood lipids [8].

1.2 Tigernut

Tiger nut "*Cyperusesculentu slativum"* is an underutilized tuber of family *Cyperaceae,* which produces rhizomes from the base of the tuber that is somewhat spherical. It is a tuber that grow freely and is consumed widely in Nigeria, other parts of west Africa, east Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula [9]. The tiger nut milk was classified as medicinal drink due to it been highly energetic and diuretic, rich in mineral, predominantly phosphorus and potassium and also vitamins C and E [9]. Tiger nuts tubers appear somewhat long or round in shape with a dimension of 8mm to 16mm, smaller size however, are not used for human consumption. When hydrated, it is slightly harder (nut texture), but with a rather more intense and concentrated taste. Being cultivated through continuance irrigation, tiger nut has to be properly dried before storage. The drying process is completely natural,(i.e. sun drying) and the process can take up to one month. The dehydrating process ensures longer shelf life, preventing rot or any other bacterial infection securing their quality and nutritional level. Unfortunately, the dehydration process make the tiger nut skin wrinkled, a situation that limits its acceptability to some people [10]. It also yield more milk upon extraction, contains lower fat and higher protein and less anti nutritional factors especially polyphenol [11]. Recently, there is awareness for increased utilization of tigernut

(Belewu and Abodunrin, 2006) [10]. Soymilk contains isoflavones (classified as phytoestrogen) which have a chemical structure similar to the hormone estrogen and binds to the estrogen receptor in the body. There are two estrogen receptors in the body. When isoflavones attach to one, they produce estrogen-like effects, but when they attach to the other, they have an anti-estrogen effect and because of this isoflavones in soy milk is link to breast cancer. The phytoestrogens may also have negative effects on thyroid function, especially in those with thyroid disease or subclinical thyroid disease or those who are deficient in iodine. Aside from isoflavone, soy milk also containsphylates which are anti-nutrients that can block the absorption of certain minerals, like iodine, zinc, iron, magnesium, copper and chromium. If consumed a lot together with eating processed foods that contain soy, this can increase the risk of developing nutritional deficiencies.

Due to the presence of anti-nutritional compounds such as phytates and oxalates in tiger nut; these anti-nutrients have specific effects on the body. Phytates may result in reduction of calcium and iron absorption, while oxalate could result in reduction of calcium formation and also, encouraging kidney formation.

The aim of the study is to isolate *Staphylococcus aureus*found in locally produced Kunu, soy milk and tiger nut drink, also to carry out the nutritional and proximate compositions of the juices.

The objectives of the study include the following:

1. To determine the proximate composition such as: moisture content, ash content, crude fiber, crude fat, protein content, carbohydrate content.
2. To determine the mineral compositions which include: calcium, phosphorous, magnesium, iron and potassium.
3. To determine total bacteria count and faecal count.
4. To isolate and carry out microbial analysis on bacterial species

1.3 Justification of the Study

In developing Nigeria, it has not been possible to have control over processing of hawked drinks because most vendors lack the adequate knowledge of food processing and adequate handling practices. As such, there is likely to be a high risk of chemical and microbial contamination. A large number of bacteria have been reportedly implicated in food spoilage as they used the carbohydrate content of food for undesirable fermentation processes [12, 5]. Kunu, soymilk and tiger nut are rich beverages and food products rich in fiber, protein and Vitamin and a substitute for cow milk and other source of protein, cheaper than other source of protein. There is need to ensure that the milk is hygiene prepared, free from bacteria or other spoilage organism. Therefore, it becomes very necessary to conduct this research to determine the bacterial load, proximate and mineral composition of these drinks in Awka, Anambra state.

Significances of the Study; Milk is an excellent source of most nutrients. In developing countries, the cost of diary milk is prohibitive. The high cost of milk in developing countries has led to the development of alternative source of milk from plant materials. Because of its underutilized less expensive and rural nature, it is hardly processed commercially and since it is processed locally, heat processing treatment like pasteurization, to combat pathogenic microorganisms in the juices.

The significant is to enable producers to improve hygienic condition handling of the drinks and a good knowledge of safe food, also to enlighten the public of various pathogenic organisms present hence increasing health awareness on the dangers of drinking the juices. This study focuses on the bacterial strain of *Staphylococcus aureus* in locally produced juices (kunu, soy milk and tigernut) when poorly processed and stored, also to dictate the proximate and mineral composition of the drinks.

2. MATERIALS AND METHODS

2.1 Sample Collection

Three samples each of kunu, soy milk and tiger nut drink was purchased from Awka market and taken to the laboratory for analyses.

2.2 Method

2.2.1 Moisture content determination

The AOAC [13] method no. 945.38 was used. About 5g of the sample was weigh .into clean, dry and pre weighed crucibles. The crucibles and

their contents was dry in the moisture extraction oven at 110^{0}C for 4 hours. The samples was cool in desiccators and reweighed. The samples was dried in the oven until a constant weight is obtained.

$$\%\ Moisture\ content = \frac{Initial\ weight - weight\ of\ oven\ sample\ X\ 100}{\text{Initial weight of sample}}$$

2.2.2 Crude fat determination

Method no. 920.39A [13] was used. Approximately 5g of the air dried ground sample was weighed into a filter paper, wrapped carefully and put in the sample holder of the soxhlet extraction apparatus. A clean dry and weighed soxhlet extraction flask was half filled with N-hexane and the whole apparatus was assembled together, and the flask placed on the heating mantle and heated at 60^{0}C.

The fat was extracted for three hours. Then, the sample holder was disconnected and the extraction flask removed. The percentage fat contained was determined thus:

$$\%\ Crude\ fat = \frac{weight\ of\ flask + oil - weight\ of\ empty\ flask\ X100}{\text{Initial weight of sample}}$$

2.2.3 Crude fiber determination

Method No. 942.05 [13] was used. 2g of defatted sample was weighed into 250 ml beaker containing 200 ml of 0.125M tetraoxo-sulphate(iv) acid (Sulphuric acid). The mixture was heated in a steam bath at 70^{0}C for hours, and then allowed to cool. The cooled mixture was filtered using a muslin cloth over a Buckner funnel. The residue was washed three times with hot water to remove the acid and then put in a beaker containing 200 ml of potassium hydroxide. The mixture was heated as before over a steam bath for 2 hours. The solution was filtered and the residue washed three times with hot water. The final residue obtained was put in clean pre-weighed crucible and dried at 120^{0}C to a constant weight. The crucible with the dry sample was put in a muffle furnace and ash at 550^{0}C for 30 minutes such that the sample became ash white. Percentage fiber was calculated as followed:

$$\%\ Crude\ fiber = \frac{weight\ of\ oven\ dried\ sample - weight\ of\ ash\ X\ 100}{\text{Initial weight of sample}}$$

Method no. 955.04C called the Kjeldahl method was used [12]. This method was divided into three namely, digestion, distillation and titration.

Digestion: Approximately 0.1g of ground sample was weighed into clean dried Kjeldahl flask for digestion, and 0.1g copper tetraoxo-sulphate(iv) crystals, 0.5g sodium tetraoxosulphate(iv) crystal and 25ml of concentrated H_2SO_4 acid was added into the flask and some glass beads was added into the flask content as anti-bumping agents. The Kjeldahl flask and its content was transferred to the digesting chamber in a fume cupboard and digested. Digestion continued with constant rotation of the digestion flask until the sample changed colour (that is from black to light blue). The digestion flask was removed from the digesting chamber and allow cooling. The digest was made up to 100ml using distilled water and shaken vigorously to a homogenous solution.

Distillation: Out of the homogenous solution of the digest, 20ml was transferred into a distillation flask using a pipette. Then 20ml of 40% sodium hydroxide solution was added carefully down the side of the flask through a funnel.

Then 50ml of 2% boric acid solution was pipetted into a receiving flask and two drops of methyl red indicator added. The distillation unit was fitted such that the condenser is connected to the receiving flask with a glass tube, and the condenser cooled with constant supply of cold water from tap. Also, the tip of the glass tube was immersed in the boric acid. The distillation unit is heated on a heating mantle for 35 minutes until the pink solution of the boric acid turned blue and the volume increased to about 100ml by the distillate.

Titration: Ten millilitres of the distillate was titrated against 0.1N hydrochloric acid to a colour-less end point. A blank solution will also be titrated to get any trace of nitrogen in the blank. All the titre volumes were recorded. The percentage crude protein was calculated as follows:

%Crude protein = % Nitrogen X 6.25

2.2.4 Ash content determination

The AOAC [12] method No 942.05 was used. Clean dried crucibles was weighed on an electronic balance and 5g of sample weighed into the crucibles. The samples was dry in the oven until constant weights are obtained.

Then, the samples was transferred into the muffle furnace with a pair of tongs and ash at 550^0C 4 hours until ash was obtained. The sample was removed from the furnace and cooled in desiccators, and reweighed. The percentage ash was calculated as followed:

$$= \% \text{ Ash Content} = \frac{Weight\ of\ Ash\ X\ 100}{\text{Weight of sample (after oven drying)}}$$

2.2.5 Carbohydrate content determination

The carbohydrate content of the sample was obtained by difference, that is, as the difference between the total summations of percentage moisture, fat, fiber, protein, ash and 100%.
Carbohydrate= 100 – (% moisture + % fat + % protein + % fiber + % ash).

2.2.6 Mineral element analysis

The mineral contents of the test samples was determined by the dry ashextraction method following each specific mineral element as described by AOAC [14]. Twenty (20) grams of the samples was burnt to ash (as in ash determination and the resulting ash was dissolved in 100ml of dilute hydrochloric acid (1MHCL) and then diluted to 100ml volumetric flask using distilled water. The solution was used for the various analysis of mineral.

2.2.7 Determination of calcium

Calcium contents of the test sample was determined by the EDTA complex isometric titration. Twenty (20) ml of each extract was dispersed into a conical flask and panels of the masking agents, hydroxytannin, hydrochlorate, and potassium cyanide was added followed by 20ml of ammonia buffer (pH 10.0). A pinch of the indicator-Ferrochrome black was added and the mixture was shaken very well. It was titrated against 0.02N EDTA solution. The calcium contents was calculated using the formulae below.

$$Calcium\ \ (\boldsymbol{mg/100g}) = \frac{(Tv\ x\ 0.4008\ x\ 1000)}{\text{Vol of sample used}}$$

2.2.8 Determination of magnesium

Exactly 10ml of the sample filtrate was pipetted into 250ml conical flask after which 25ml of ammonia buffer solution was added into the conical flask and was properly mixed. Then a pinch of Erichrome black T indicator was added and titrated with 0.02N of EDTA until the colour of the solution change.

$$Magnessium\ \ (\boldsymbol{mg/100g}) = \frac{(Tv\ x\ 0.2432\ x\ 1000)}{\text{Vol of sample used}}$$

2.2.9 Determination of potassium (K)

The concentrations of potassium (ppm) was analyzed using UV- spectrophotometer at a wavelength of 766.5 nm, and the concentration in mg/100 g was calculated using the following equation:

$$Potassium\ (\boldsymbol{mg/100g}) = \frac{Concentration\ (ppm)\ x\ Dilution\ factor\ X\ 1000}{\text{Wt of Sample}}$$

2.2.10 Determination of Iron (Fe)

The concentrations of chromium (ppm) wasanalysed using atomic absorption spectrophotometer at a wavelength of 243nm and the concentration in mg/100 g was calculated using the following equation:

$$Iron\ (\boldsymbol{mg/100g}) = \frac{Concentration\ (ppm)\ x\ Dilution\ factor\ x\ 1000}{\text{Wt of Sample}}$$

2.2.11 Determination of phosphorus (P)

A 20 ml sample solution was put in a 100 ml volumetric flask. The solution was neutralized with ammonia and nitric acid solution (1:2). Twenty (20) ml of vanadate molybdate reagent was added and diluted to the mark. It was allowed to stand for ten minutes and absorbance read at 470nm in the ultra violet region and the mineral concentration in mg/100 g was calculated using the following equation:

$$Phosphorus\ (\boldsymbol{mg/100g}) = \frac{Concentration\ (ppm)\ x\ Dilution\ factor\ x\ 100}{\text{Wt. of Sample}}$$

2.3 Microbial Analysis

2.3.1 Preparations of culture media

(a). Nutrient agar (NA): Nutrient Agar was prepared by dissolving 28 g of nutrient agar powder in 1000 ml of distilled water in a clean flask. The mouth of the flask was plugged with

non-absorbent cotton wool wrapped with aluminum foil paper that was extended up to the neck of the flask. The flask was placed on a bunsen flame and allows to boil and mix completely. It was sterilized in an autoclave at 12IOC for 15 minutes and allowed to cool to 450C and aseptically dispensed into Petri dishes. Nutrient agar was used for the total bacterial aerobic plate count.

(b). Macron key Agar (MA): This agar was prepared by dissolving bile salt, Then 48.5 g of the powder was dissolved in 1000 ml of distilled water. The pH was adjusted to 7.8. It was autoclave at 121°C for 15 minutes and allowed to cool to a temperature of 45 - 50°C before pouring into plates. This was used to determine coliforms as described by Cheesbrough [15]. This is a selective and differential media designed to isolate and differentiate organism based on their ability to ferment lactose as described by Sebastia et al. [16].

(c) Corn meal agar (CMA): Corn meal agar was used to isolate yeast and it's prepared by dissolving 17 grams of corn meal powder in a 1000 ml of distilled water. The mixture was heated gently to dissolve the medium completely. 1 % of polysorbate was added and sterilized in autoclave at 121°C for 15 minutes. It was cool at room temperature before pouring into petri dish containing 1ml of the sample as described by Zumbes et al. [17].

(d). Potatoes dextrose agar (PDA): The medium PDA was prepared by using 39 grams of potatoes dextrose agar powder. It was dissolved in 1000 ml distilled water. It was heated to boiling, in order to get mixed completely. Then sterilized in an autoclave at 121°C for 15minutes, this particular media was used to this particular media.

(e). Mannitol salt agar (MSA): The medium was prepared by dissolving 108 grams of mannitol salt agar in 1000 ml of distilled water, after which it was allowed to stand for 10 minutes, swirled to dissolve properly. The mixture was sterilized in an autoclave at 121°C for 15minutes and allowed to cool to a temperature of 45°C before pouring into the appropriate petri dish as described by Fowoyo [18]. Mannitol salt agar was used to determine and enumerate the bacteria *Staphylococcus auerus*.

2.4 How to Identify Bacteria Strain

(i). **Identification of microbial isolate**: Identification of the microbial isolate was performed using classical methods based on their morphological and biochemical characteristic with reference to systematic manual of bacteriology described by Cheesbrough [15].

(ii). **Gram staining technique**: Gram staining reaction has the wide application that is capable of distinguishing virtually all bacteria into one of two large group — gram positive or gram negative. Smear of each isolate was made on the slide and heat fixed. Primary stain (crystal violet) was added in drops. Lugols iodine was added for 45 seconds decolorized with acetone and washed with water. It was then air dried examined at Xl00 under oil immersion as described by Bello et al. [19] Positive gram staining appears purple and negative grams staining appeared pink.

(iii). **Motility**: The medium used for motility test (agar with concentration of 0.5%) was inoculated with test organism. A stab of each inoculate was made at the center of each tube. The tube at 37oC was incubated for 24 hours. A diffused growth at the place of inoculation was considered as positive and restricted growth was considered as negative.

(iv). **Citrate Test:** The citrate test was performed by inoculating into organic synthetic medium in which sodium citrate is the only sources of carbon and energy. In sodium citrate broth (Koser's citrate medium), the presence of growth (turbidity) is a positive test result.

(v). **Indole Production:** Indole is produced in triptone broth by the enzyme of certain organisms. Triptone broth is rich in amino acid tryptophan which can be used by some bacteria as source of carbon, energy as well as nitrogen. Tryptophan is degraded to indole pyruvic acid and ammonia by some microorganisms. A loopful of test culture (Twenty four hour old) was inoculated into the triptone broth and incubated for two days. Into six milliliters of culture broth a three milliliters of Kovac's reagent was added from aqueous layer, colour change to red is a positive test.

(vi). **Urease Test:** Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by means of the enzymes urease. This ability was tested for using Christensen medium with heavy inoculation of the

isolates was made and the agar slants in tube was observed after 24 hours and then incubated further for hours. 'This test was used to identify coli forms as reported by Musa and Hamm [20].

(vii). **Coagulase test:** The use of blood plasma is being introduced in coagulase test. A loop full of human plasma was added to culture isolate on a slide. Positive isolate gave agglutination reagent with plasma. Test was also carried out at 37°C for 24 hours' positive tubes showed coagulation of the plasma in the tube.

(viii). **Catalase test:** Catalase test was carried out using a drop of hydrogen peroxide. 2 ml of 3% hydrogen peroxide (H202) was placed in a clean test tube. A sterile wire loop was used to pick a colony of the test organism and mixed with 2 ml of 3% hydrogen peroxide (H2O2) in the test tube and observed for the production of gas bubbles which indicates a positive reaction. This test was used to identify *Staphylococcus aureus*.

(ix). **Oxidase test:** A few drops of kova's reagent were added to piece of filter paper on a petri dish. The bacteria isolates were then smeared on the filter paper with a glass rod. The paper was observed. Positive result gave a dark purple color while negative result showed no color change. This test was used to identify coliforms. As reported by James [21].

3. RESULTS AND DISCUSSION

Moisture content of kunu 81.00%, % ash content is 1.85. This value was higher than 0.20% obtained by otaru et al. [22], but the results however agree with 2.00 to 3.00% obtained by Innocent et al. [4]. % content of crude fat, crude fiber, crude protein and carbohydrate were 0.81, 0.53, 1.85 and 13.96 respectively. Essien et al. [23] reported that loss of protein during processing of the drinks may be responsible for the low protein content observed. Different cereal types have abilities to contribute to the ash content of kunu- zaki as a result of the differences in their ash compositions. The high carbohydrate content of kunun-zaki indicates a good source of energy needed for human activity [24].

Tigernut contain 84.00% moisture, 3.08% ash, 0.14% fiber, 2.70% protein, 1.05% fats and 11.03% carbohydrate. Soy milk contain 86.00 moisture, 2.22% ash, 0.07% fiber, 4.40% protein, 1.37% fat and 5.94% carbohydrate.

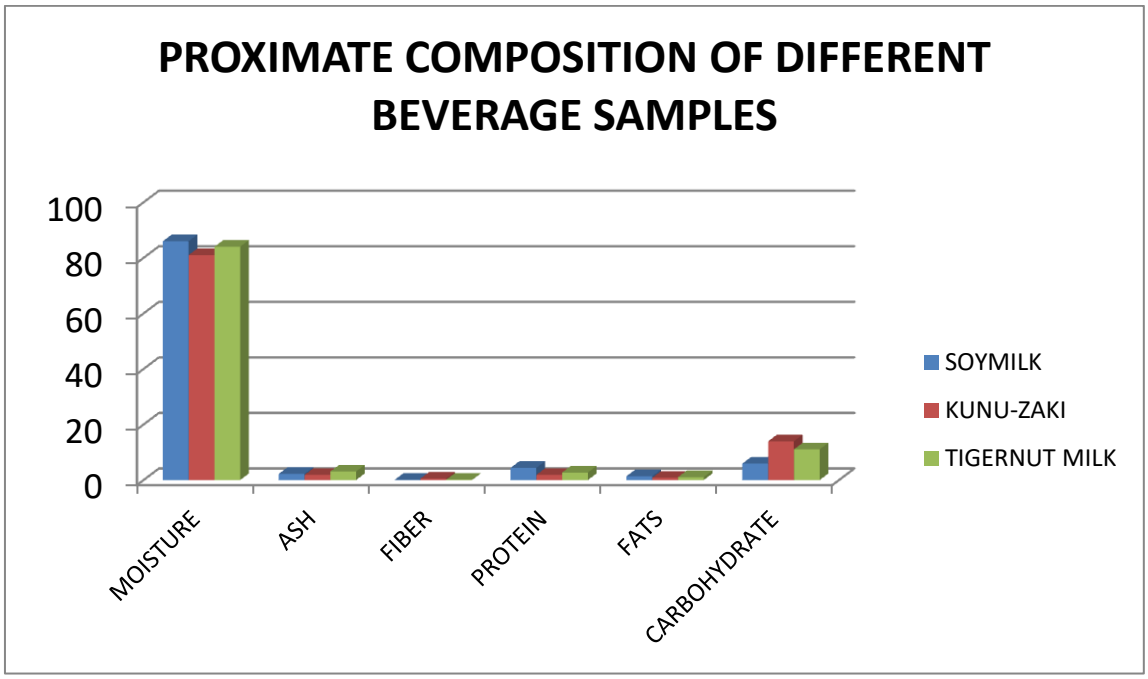


Fig. 1. Proximate composition of different beverage Sample

Table 1. Proximate Composition of the Drinks (Soy Milk, Kunu and Tiger Nut)

Sample	Moisture	Ash	Fiber	Protein	Fats	Carbohydrate
Soymilk	86.00	2.22	0.07	4.40	1.37	5.94
Kunu-zaki	81.00	1.85	0.53	1.85	0.81	13.96
Tigernut milk	84.00	3.08	0.14	2.70	1.05	11.03

Table 2. Mineral Analysis Results of Kunu, Soy Milk and Tiger Nut

Sample	Calcium	Iron	Potassium	Magnesium	Phosphorus
Soymilk	127.89	0.85	17.60	147.00	11.37
Kunu-Zaki	217.90	2.37	113.00	106.05	20.00
Tigernut Milk	221.00	2.83	135.00	175.00	75.10

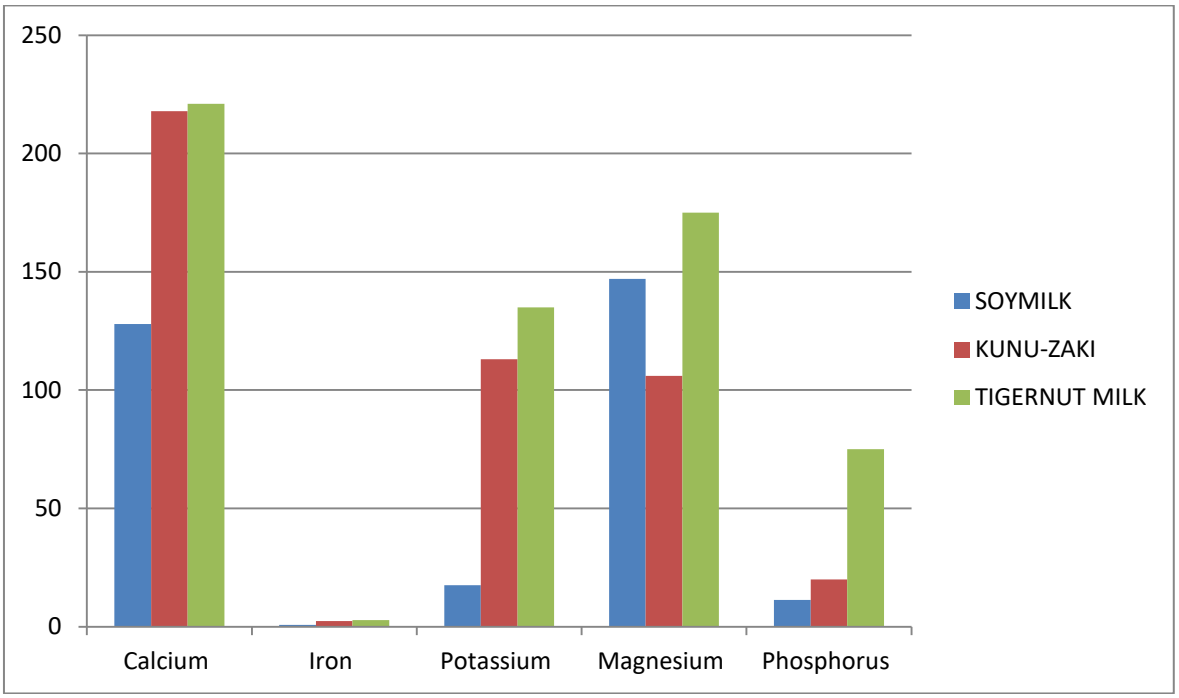


Fig. 2. Graphical representation of mineral analysis of the drinks

Table 3. Microbial analysis

Sample	Total Bacterial count (cfu/ml)	Faecal Coliform counts (cfu/ml)
Soymilk 1	0.90 x 106	5.90 x 104
Kunu-Zaki1	0.60 x 106	5.50 x 104
Tigernut Milk 1	0.50 x 106	5.13 x 104
Soymilk 2	0.70 x 106	5.10 x 104
Kunu-Zaki2	1.35 x 106	3.37 x 104
Tigernut Milk 2	1.59 x 106	3.50 x 104
Soymilk 3	1.97 x 106	3.00 x 104
Kunu-Zaki3	1.90 x 106	3.50 x 104
Tigernut Milk 3	0.40 x 106	1.15 x 104

The ash content is an inorganic residue remaining after the removal of water and organic matter by heating in the presence of oxidizing agents. This gives a measure of the total amount of minerals in a food. Ash in dairy product is an important source of many minerals and vitamins and in low calorie density.

The increase in the mineral content in the samples could therefore justify the need to enrich

Table 4. Morphological and biochemical characteristics of isolates

Parameters	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7
Colony Characterization	Milkish irregular shape with flat elevation	Pinkish circular with flat elevation	Yellowish circular with flat elevation	Whitish irregular shape with flat elevation	Yellowish irregular shape with flat elevation	Whitish irregular shape with flat elevation	Yellowish irregular shape with flat elevation
Cell characterization	Coci in clusters	Long rods in singles	Short rods in singles	Rods in clusters	Cocci in clusters	Rods in clusters	Cocci in clusters
Gram's Test	Positive	Negative	Negative	Negative	Positive	Negative	Positive
Motility Test	Negative	Negative	Negative	Positive	Positive	Positive	Positive
Catalase	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Coagulase	Negative	Negative	Negative	Negative	Positive	Negative	Positive
Citrate	Negative	Negative	Negative	Negative	Positive	Negative	Positive
Indole	Negative	Positive	Positive	Negative	Positive	Negative	Positive
Oxidase	Negative	Negative	Negative	Positive	Negative	Positive	Negative
Urease	Positive	Positive	Positive	Negative	Positive	Negative	Positive
Probable organism	*Klebsiella spp*	*Salmonella spp*	*Shigella spp*	*E- coli*	*Staphylococcus spp*	*Vibrio sp.*	*Pseudomonas spp*

Table 5. Antibiotic susceptibility pattern of the bacterial isolate

		Antibiotics sensitivity profile											
Sample	**Isolates**	**CN**	**S**	**LC**	**CPX**	**RX**	**E**	**NOR**	**CH**	**OFX**	**PEF**	**AU**	**SXT**
SOYMILK	*Klebsiellasp.*	S	S	R	S	R	I	R	R	R	R	R	I
	Vibrio sp.	S	S	I	S	I	R	S	S	S	R	R	R
	Staphylococcus sp.	R	R	R	R	R	R	R	R	S	R	R	R
KUNU-ZAKI	*Staphylococcus sp.*	R	R	S	I	R	R	R	R	I	S	R	R
	Escherichia coli	S	R	S	S	R	R	S	I	S	S	R	S
	Klebsiellasp.	S	R	R	R	R	R	R	R	S	S	R	R
	Salmonella sp.	R	S	R	S	R	R	I	R	S	S	R	S
	Vibrio sp.	I	R	R	S	R	R	S	R	S	I	R	R
TIGERNUT MILK	*Vibrio sp.*	R	S	R	I	R	R	S	R	S	S	R	R
	Shigellasp.	R	S	R	R	R	R	R	R	S	R	R	R
	Klebsiellasp.	S	S	I	S	R	S	S	S	S	S	S	S
	Vibrio sp.	S	R	R	I	R	R	I	S	S	S	R	R

***N/B: R** = Resistant**, I** = Intermediate**, S** = Susceptible; **CN** - Gentamycin; **S** – Streptomycin; **LC** –Lincocin; **CPX**-Ciprofloxacin; **RX** – Rifampicin; **E** –Erythromycin**; NOR** - Norfloxacin; **CH**-Chloramphenicol; **OFX**-Ofloxacin; **PEF** -Pefloxacin; **AU**-Augumentin; **SXT**-Cotrimoxazole*

the beverage with source that are rich in other nutrients lacking in cereals normally adopted in its production [24]. Minerals are of great importance in diet as they play important roles in body metabolism.

Potassium is an important mineral that conduct electricity in the body along with sodium chloride, calcium and magnesium. It is crucial to heart functions and plays a key role in skeletal and smooth muscle concentration. Magnesium works as an enzyme cofactor. It helps in the formation of DNA and RNA. It regulates the cholesterol production in the body. The body use 99 percent of its calcium to keep bones and teeth strong and healthy. It supports skeletal structure and function. The rest of the calcium in body plays a key role in cell signaling, blood clotting, muscle contraction and nerve functions. From the analysis, tiger nut is highly rich in mineral nutrients. Iron is required for growth and development. It also plays a central role in many biochemical processes in the body. These include oxygen transport and storage, assisting with immunity and contributing to enzyme systems. Phosphorus plays an important role in how the body uses carbohydrates and fats. It is also needed for the body to make protein for the growth, maintenance, and repair of cells and tissues.

From the graphical representation above, it shows that tigernut drunks is highly rich in minerals as it has the highest values for all. The drink with the least minerals is soy milk.

The total bacteria counts (CFU/ml) of soy milk, kunu and tigernut ranged from (0.70×10^6 to 1.97×10^6), (0.60×10^6 to 1.90×10^6), (0.40×10^6 to1.59×10^6) respectively. The faecal bacteria count (CFU/ml) of soy milk, kunu and tigernut ranged from (3.00×10^4 to 5.90×10^4), (3.37×10^4 to 5.50×10^4), (1.15×10^4 to 5.13×10^4) respectively. The results indicate that fresh kunu presented a high bacteria count after 24hrs of incubation. The high colony count is an indication of spoilage as a consequence of either poor hygiene or poor quality of cereals and water used. The presence of coliform bacteria in these drinks as determined in this research was of public health concern because teaming populace, especially students, relies on these drinks as cheaper alternative to the bottled soft drink.

The bacterial isolate are seven and it include: *Staphylococcus spp*, *Vibrio spp,Pseudomonas spp, E. coli, Shigellaspp, Salmonella spp and Klebisella spp.*

The presence of *E. coli* in kunu indicates faecal contamination and may have serious health implications. *Pseudomonas* and *Klebsiella spp* have been implicated in the spoilage of food and beverages. Their presence in kunu, soymilk and tigernut is undesirable. There is then the need to maintain adequate hygienic conditions during processing and preparation of the beverages to eliminate these microbial contaminants and to improve on the quality of the final product. There is also the need to employ adequate preservative measures to improve the shelf-life of the beverages.

4. CONCLUSION

The result of the study provides information on the nutritive values of all drinks samples. Soy milk has the highest moisture, fat and protein contents, kunu-zaki is highly rich in carbohydrate and fiber whereas tiger nut has a higher amount of ash content. Tiger nut drink is extremely rich in minerals from the graphical representation. The microbial content of these hawked beverages were high and were contaminated with microorganisms which are potentially pathogenic to man. This possess a threat to the general public, as these contaminants has ability to cause varying level of diseases, ranging from food borne illness and food poisoning due to *staphylococcus aureus*. The presence of these isolated organisms in the beverages analyzed could serve as an indicator for the need to promote awareness about possible health hazards that could arise due to handling and processing.

5. RECOMMENDATION

(i). It is recommended that local beverages should be adequately fortified so that the nutrients loss during processing would be replaced.

(ii). Regulatory agencies should intervene by setting standards in acquisition of raw material, production techniques as well as health status of personnel involved in the production process of non-alcoholic beverage widely consumed in Nigeria.

(iii). Education of the manufacturers and provision of basic facilities will greatly improve non alcoholic beverage drinks quality and safety.

(iv). More work should be carried out on the antibacterial resistance of all beverages.

COMPETING INTERESTS

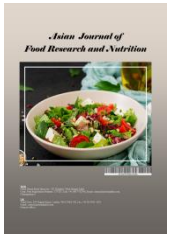
Authors have declared that no competing interests exist.

REFERENCES

1. Akoma O, Jiya EA, Akunmka DD, Mshila E. Influence of malting on the nutritional characteristics of kunun-zaki. African Journal of Biotechnology Linked presence of these Bioactive Compounds in Plant Materials to Antimicrobial Activity. 2006;5(10):996 – 1000.
2. Amusa NA, Ashaye OA. Effect of processing on nutritional, microbiological and sensory properties of kunun-zaki (a sorghum based non-alcoholic beverage) widely consumed in Nigeria. Pakistan Journal of Nutrition. 2009;8 (3):288– 292.
3. Adebayo GB, Otunola GA, Ajao TA. Physicochemical, microbiological and sensory characteristics of kunu prepared from millet, maize and guinea corn and stored at selected temperature. Adv. J. Food Sci. and Technology. 2009;2(1):41-46.
4. Innocent OO, Mariam YO, Blessed K, James TW. Microbial evaluation and proximate composition of kunuzaki, an indigenous fermented food drink consumed predominantly in Northern Nigeria. International Journal of Food Safety. 2011;13:93-97.
5. Amusa NA, Ashaye OA, Aiyegbayo AA, Oladapo MO, Oni MO, Afolabi OO. Microbiogical and nutritional quality of hawked sorrel drinks (soborodo), the Nigerian locally brewed soft drinks widely consumed and notable drinks in Nigeria; 2005.
6. UNUCED. Soybeans. United Nations Conference on Trade and Development. 2016;25:1-25.
7. Ganshrao DC. Design and development of roaster for production of soyanut. Msc, College of Agricultural Engineering and Technology, India; 2016.
8. Kohli D, Kumar S, Shuchi U, Mishra R. Preservation and processing of soymilk. International Journal of Food Science and Nutrition. 2017;2(6):66-70.
9. Abaejoh R, Djomdi I, Ndojouenkeu R. Characteristics of tigernut (*Cyperus esculentus*) tubers and their performance in the production of a milky drink. Journal of Food Processing Preservation. 2006; 30:145-163.
10. Belewu MA, Belewu KY. Comparative physico-chemical evaluation of tigernut, soybean and coconut milksources. International Journal of Agricultural Biology. 2007;9:785- 787.
11. Okafor JN, Mordi JI, Ozumba AU, Solomon HM, Olatunji O. Preliminary studies on the characterisation of contaminants in tiger nut (yellow variety). In: Proceedings of 27th Annual Conference and General Meeting of Nigerian Institute of Food Science and Technology Kano. 2003;210-211.
12. Ojokoh ST, Adetuyi AA, Akinyosoye FA, Oyetayo VO. Fermentation studies on Roselle (*Hibiscus sabdariffa*) calcyces neutralized with Trina Journal of Food Technology. 2002;7:75-78.
13. AOAC. Official Method of Analysis.16th Edition, Association of Official Analytical, Washington DC; 2002.
14. AOAC. Official method of Analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24; 2005.
15. Cheesebrough M. District Laboratory practice in Tropical countries Cambridge University press, Cambridge, UK. 2004;62-70.
16. Sebastia NE, lShenawy MJ, Soriano JM. Assessment of microbial quality of commercial and house made tiger-nut beverages. Letters in Applied Microbiology. 2012;5(54):299-305.
17. Zumbes JH, Dabo AD, Dakul DA, Afolabi SA, Dapiya MS. Enter pathogenic bacterial contamination of some ready to eat foods sold in Jos metropolis. Nigeria Indian Journal of Applied Research. 2014;4(7):456-458.
18. Fowoyo PT. Microbiological quality assessment of air contamination of vended food sold in the main market in Lokoja, Kogi state, Nigeria. Research Journal or Biological. 2012;7(9-12):355-360.
19. Bello OO, Bello TK, Fashola MO, Oluwadan A. Microbiological quality of some locally produced fruit juice in Ogun state. South West Nigeria Journal of Microbiology Research. 2014;2(1):001-008.
20. Musa AA, Hamza A. Comparative Analysis of locally prepared KununAya Tiger nut

milk consumed by student in Kaduna slate university Kaduna Nigeria. Science World Journal. 2013; 8(2).
21. James MJ. Modern food microbiology 3rd (Edn.), Tafa MC, Graw Hill Publishers Co. ltd. 2001; Pp 1 10-125.
22. Otaru AJ, Ameh CU, Okafor JO, Odigure JO, Abdulkareem AS. Development, carbonation and characterization of local millet beverage (Kunu). International Journal of Computational Engineer Research. 2013;3(4):80-86.
23. Essien E, Monago C, Edor EA. Evaluation of the nutritional and microbiological quality of Kunun (a cereal based non-alcoholic beverage) in Rivers State, Nigeria. The Internet Journal of Nutrition and Wellness. 2011;10:1-10.
24. Iwe MO. Handbook of Sensory Methods and Analysis. Rejoint Communication Services Ltd Uwani Enugu. 2002;40-83.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97812



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 48-57, 2023; Article no.AJFRN.98483

Nutritional Quality of Complementary Porridge for Feeding Children Aged 6-24months from Selected Local Food Ingredients

Wilson Martha [a*] and Alex Wenaty [a]

[a] *Department of Food Science and Agro-Processing, School of Engineering and Technology, Sokoine University of Agriculture, P.O. Box 3006, Chuo Kikuu, Morogoro, Tanzania.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/98483

Original Research Article

Received: 07/02/2023
Accepted: 09/04/2023
Published: 14/04/2023

ABSTRACT

Flours from yellow maize, pumpkin seeds, soybeans and carrots were blended in different proportions. Formulated flour samples were; MPSC1 (55:15:20:10), MPSC2 (60:17:15:8), MPSC3 (65: 20:15:5) and control samples were MP (75:25) and MS (75:25). Extruded flour samples were assessed for proximate, minerals (magnesium, iron, zinc) and vitamin A content and data obtained were analyzed statistically by using One way ANOVA. Protein content results of the formulated flour samples ranged from 18.45 g/100g in to 14.08 g/100g. Vitamin A content in formulated flours ranged from 324.5 µg/100g to 21 µg/100g. Zinc amount, results ranged from 22.58 mg/100g to 9.41 mg/100g. Magnesium amount, results ranged from 281.33 mg/100g to 31.08 mg/100g. Iron content from the formulated samples ranged from 13.22 mg/100g to 45.54 mg/100g. Based on the targeted nutrients of interest of this study results showed that, soybeans contributed to protein level, carrots contributed to high beta carotene amount, while pumpkin seeds contributed to zinc, magnesium and iron.

**Corresponding author: E-mail: marthasebastian411@gmail.com;*

Keywords: Yellow maize; formulation; proximate composition; minerals.

1. INTRODUCTION

"Around the age of 6 months, an infant's need for energy and nutrients starts to exceed what is provided by breast milk alone, and complementary foods are required to meet those needs. An infant of this age is also developmentally ready for other foods" [1]. "This transition is referred to as complementary feeding. If complementary foods are not introduced around the age of 6 months, or if they are given inappropriately, an infant's growth may falter" [2].

According to PAHO/WHO, 2003 infant's complementary food should be timely, be introduced when the need for energy and nutrients exceeds what can be provided through exclusive breastfeeding. Adequate, able to provide sufficient energy, protein and micronutrients to meet a growing child's nutritional needs. Safe, should be hygienically stored and prepared, and fed with clean hands and clean utensils. And lastly they should be properly fed meaning that they are given consistent with a child's signals of satiety and appetite, and that meal frequency and feeding are suitable for age.

"Tanzania's complementary feeding practices are suboptimal as they do not meet WHO recommended complementary feeding indicators such the introduction of solid, semi-solid, and soft foods, a minimum level of nutritional diversity, a minimum number of meals per day, and a minimal standard of diet"[3].

"In developing countries, a high rate of malnutrition in children under the age of five is linked to the selection of complementary foods and inappropriate feeding practices" [4]. Most of the families from several developing countries cannot afford the high cost of fortified complementary foods because of poverty [5]. As a result, many families rely on low quality, inadequately processed traditional complementary foods for their children. Because of this, protein energy malnutrition is a significant issue for infants in developing countries [2].

"In this study locally sourced raw materials were used for the formulation of complementary foods for feeding children. Several materials ranging from cereals, plant protein sources, as well as vegetables have been found to be highly nutritious and were able to serve as cheap substitutes for diet formulation" [6]. This study also aimed at production and analyzing nutritional quality of formulated complementary flour made from yellow maize, pumpkin seeds, soybeans and carrots.

"Yellow maize (*Zea mays L*) contains about 72% starch, 10% protein, 6% oil, 4% fat and sugar 3% and supplying an energy density of 365 Kcal/100 g" (Ntloko, 2020). "Maize provides many of the B vitamins and essential minerals along with fiber, also yellow maize has a high amount of vitamin A (carotenoids) but lacks some other nutrients, such as vitamin B_{12} and vitamin C, and is, in general, a poor source of calcium, folate, and iron. Fortification of maize flour and cornmeal with iron and other vitamins and minerals has been used to improve micronutrient intake and prevent iron deficiency, especially to countries where anemia and iron deficiency are considered moderate or severe public health problems" (Ranum et al*.,* 2014).

"Pumpkin seeds have an exceptional number of vitamins and minerals, particularly nutrients that are essential for baby's development, like vitamin K, copper, iron, and magnesium" (Gharibzahedi, 2017). "They are also one of the richest plant sources of zinc, a commonly deficient nutrient in young children" (Revathy et al*.,* 2013).

"Soy foods can offer several important macronutrients and micronutrients to the diets of children such as protein, essential fatty acids, calcium, potassium, and folate" (Anim et al*.,* 2013).

Soybeans are a better source of vitamin B-12, which is an excellent source of antioxidants such as phytosterone and isoflavones. Furthermore soybean is also a good source of some micronutrients such as potassium, phosphorus, calcium, magnesium, and iron thus it can be used to reduce the effect of malnutrition to children (Modgil, 2021).

A root vegetable known as a carrot (*Daucus corota*), it has a thin, conical form and is often orange in color. They include a lot of vitamins, minerals, fiber, and antioxidants. Carrots are naturally sweet, high in fibre, and a fantastic source of vitamin A (Boadi et al*.* 2021). "Vitamin A is vital for vision as it forms part of a protein that absorbs light in the eye. Additionally stimulates cell proliferation, which is necessary

for heart, lung, and kidney health. Beta-carotene is an antioxidant that converts to vitamin A. It is widely accepted that beta-carotene plays a crucial role in fighting against harmful free radicals in the body" (Badu, 2021).

Extrusion cooking was used in the production of complementary porridge because is one of the most popular methods for processing food since it produces huge amounts of food with greater nutrient retention over a longer period of time while also eradicating dangerous microorganisms and antinutrient [7]. Also extrusion process reported to improves the protein digestibility and iron bioavailability of cereals through reductions in antinutritional factors such as tannins or phytates [8]. Extruders have grown increasingly specialized for culinary applications since extrusion cooking creates a variety of foods [9]. It is important to regulate the extrusion process operations and have knowledge of the impacts of operational factors, such as temperature, screw speed, and feed moisture content so as to get products with a variety of required physicochemical characteristics [10].

One of the most ecologically friendly and energy-efficient methods for a variety of food items is extrusion processing, which has gained popularity over time [11]. Additionally, extrusion may be used to create wholesome foods that are tailored to social demands in order to solve issues like malnutrition and food insecurity [12].

Low cost indigenous and underutilized legumes that can be processed and appropriately combined with widely accessible carbohydrate sources will provide relatively affordable and nutrients dense complementary foods that will help to minimize malnutrition and enhance children's nutrition in order to reduce these issues [13].

2. MATERIALS AND METHODS

2.1 Raw Samples

Yellow maize, soybeans, pumpkin seeds, and carrot were utilized as raw materials to make composite flour. These ingredients were obtained at Chief Kingalu Market in the Morogoro municipality. These raw materials were chosen because they were rich in the desired nutrients which were vitamin A precursor (beta carotene), Zinc, Iron, Magnesium and Protein. In addition these foods are also readily available and affordable locally are grown across Tanzania. The nutritional analysis of the composite flours were done at Sokoine University of Agriculture in the laboratory of Department of Food Science and Agro-Processing.

2.2 Preparation of Yellow Maize, Pumpkin Seeds, Soybeans and Carrots Composite Flour

Yellow maize were sorted, washed then sundried and milled into fine flour with particle size 358 to 17µm, then packed into clean sealed polyethylene bags because they are tear resistant and restricts the passage of water through it. Package yellow maize flour was stored at room temperature of 23℃ to 27℃.

Pumpkin seeds were sorted, washed, soaked in water for one day for sprouting so as to convert the stored carbohydrate to consumable and for softening to ease the milling process. The seeds were roasted in the oven at 150 ℃/45min then dried at 37 ℃ in an incubator [14]. Then dried pumpkin seeds were milled into fine flour, packed into clean sealed poly ethylene bags and stored at room temperature of 23℃ to 27℃.

Soy beans were sorted and washed then boiled for 30 minutes to remove anti nutritional factor such as phytate (Abemacha, 2020), thereafter the soybeans were sprayed on the tray then roasted in the oven for at 110 ℃/80min, and then dried soybeans were milled into fine flour then packed into clean sealed polyethylene bags, and stored at room temperature. Carrots were washed with clean water, then scraped, grated and dried at 50 ^{0}C/8hrs. Then the dried carrots were blended and sieved into fine flour. The flour was packed and sealed with food grade polyethylene bags for analysis.

2.3 Formulations of Composite Flour

Flours from yellow maize, pumpkin seeds, soybeans and carrots were blended in different proportions as presented in Table 1, to develop varieties of complementary flour of composite mixtures. However, in this study, NutriSurvey [15] software was utilized to design and assess complementary flour blends. In order to prevent the issue of malnutrition during the early stages of child growth, these formulations developed must provide at least half of the recommended daily intake of the targeted nutrients, which are protein at 15 mg, 210 g of vitamin A, 3 mg of

iron, 2.5 mg of zinc, and 54 mg of magnesium for children aged 6 to 24 months.

2.4 Extrusion Cooking Process

Extrusion cooking was carried out in the Bio process engineering laboratory at Sokoine University of Agriculture, using a twin screw extruder where by the pre-conditioned feed mixture was measured into the extruder by a twin-screw volumetric feeder. The extrusion process was done under the following conditions: screw speed of 30.45 rmp, feeding rate of 8.20 kg/hr and barrel temperature was set at 62 °C and 109 °C in the first and second zones respectively [12]. The extruded materials were collected, cooled to room temperature under natural convection conditions, milled into fine flour using milling machine then, cooled, packed in polythene bags and stored at room temperature prior to porridge preparation and analysis.

2.5 Proximate Composition

Proximate composition of the flour formulations were done to determine the amount of macronutrients present in the flours. This involved the analysis of crude protein, crude fat, ash, moisture and carbohydrates. All determinations were carried out in triplicates.

2.5.1 Crude protein

Crude protein content of the flour samples was determined using the micro-Kjeldahl method 920.87 [16]. The procedure was divided into three steps namely digestion, distillation and titration (Nielsen, 2010). The dried sample (0.5 g) was weighed and transferred into digestion tubes; 0.6 g of catalyst (mixture of 10 g K_2SO_4, 0.5 g $CuSO_4$) and 6 mL of concentrated H_2SO_4 were added to each tube with a sample. Samples were digested using Tecator digestion system 40 (Model 1016 digester, Sweden) for 3 hours to obtain a clear greenish solution. The digest was cooled and mounted in the distillation unit (Foss Tecator, Model 2200 Kjeltec auto distilling unit, Sweden). The distilled water, 70 mL was added to the digest followed by 70 mL of 40% NaOH and steam distilled for 4 min. The distillate, 50 mL was collected in conical Erlenmeyer flask containing 25 mL of 4% boric acid. The distillate was thereafter titrated with 0.105 g/100 mL hydrochloric acid. The blank volume was carried out and 0.04 mL obtained. Titration equation was; $(NH_4)_2SO_4$ +2NaOH+ H_2O $NaSO_4$+NH_3+H_2O

$$\%Nitrogen= \frac{14.01x(\text{titre}-\text{blank})\text{mL xconcentration of acid in n/mol}}{\text{weight of sample(g)x10}} x10 \ldots \quad \text{(i)}$$

$$\% \ CP = \%Nitrogen \ x \text{ nitrogen conversion factor } 6.25\ldots \quad \text{(ii)}$$

2.5.2 Crude fat

Total fat was determined by using Soxhlet extraction official method 945.87 [16]. The dry sample (5 g) was placed into the extraction thimble and assembled to the Soxhlet apparatus. The petroleum ether (60 mL) of was used for continuous reflux for 55 min in three phases, the boiling phase for 15 min, the fat extraction phase for 30 min and the petroleum ether recovery phase for 10 min. Petroleum ether was then recovered by evaporation. Pre-weighed cups containing fat were dried in an oven at 105 ^{0}C for 30 min to evaporate any remaining petroleum ether, cooled in a desiccator for 20 min and weighed. Percentage fat was calculated by using the formula:

$$\%Fat = \frac{Weight\ of\ crude\ fat\ (g)}{Weight\ of\ dry\ sample(g)} x100\ldots \quad \text{(iii)}$$

2.5.3 Crude fibre

Crude fibre was determined by using AOAC [16] official method 920.86. Ankom fibre analyzer (Model ANKOM 220, USA) was used for the determination of crude fiber. Flour sample of 1.0 g was digested in the fiber analyzer by dilute sulphuric acid (0.125 M H2SO4) for 30 min and washed with hot water. The residues were then digested by dilute alkali (0.125 M KOH) for 30 min and washed by hot water. Digested residues were dried in the oven 105^{o}C for 5 h, cooled and weighed. The residues were then placed in muffle furnace and incinerated at 550^{o}C/2 hrs, cooled and weighed again. Total fiber content was taken as the difference between the residues before and after Incineration:

$$\%\ C.F = \frac{(Weight\ of\ sample\ residues\ before\ incineration - Weight\ after)g}{Weight\ of\ dry\ sample\ taken\ for\ determination(g)} \times 100\ldots \quad \text{(iv)}$$

Table 1. Formulations for complementary flour

Samples	Yellow maize (%)	Pumpkin seed (%)	Soybean (%)	Carrot (%)
MPSC1	55	15	20	10
MPSC2	60	17	15	8
MPSC3	65	20	10	5
MPS	75	25	-	-
MS	75	-	25	-

2.5.4 Ash content

Ash content was determined according to AOAC [16], method 923.03. Five grams of dry sample was oven dried at 105 ^{o}C /24 h. The weight of crucible and dried sample were recorded. The dried samples in crucibles were incinerated in a muffle furnace at 550 ^{0}C for 3 h, grey ash was obtained. Ash content was calculated as the difference between the weight of sample before and after incineration Percentage ash was calculated from the relationship:

$$\%Ash\ (DM) = \frac{Weight\ of\ ash\ (g)}{\substack{weight\ of \\ dry\ sample\ (g)}} \times 100\ldots \quad (v)$$

2.5.5 Carbohydrate content

Carbohydrate was calculated as a percentage difference using the formula:

% Carbohydrate = 100 % – (% protein + % crude fiber + % crude fat + % Ash)… (vi)

2.6 Vitamin A

Beta carotene determination was done according to Delia and Mieko [17] whereby 5g of porridge triplicate samples in a test tube were measured and homogenized 4 times using 50mL proportions of cold acetone before extraction. The extracts were then transferred into the separating funnel containing petroleum ether (40-60 ^{o}C), followed by a thoroughly washing with about 300mL of distilled water until the extracts is acetone free. During the washing process, the distilled water was kept along the wall of the glass separating funnel to avoid formation of emulations (water stones) in the carotenoid extracts. Then washed sample was allowed to pass through anhydrous sodium sulphate to dry it. The dried carotene extracts were collected into a clean and dry volumetric flask. Beta carotene stock standard solution with the concentration of 100 µg/mL was prepared. This stock solution was diluted to obtain 0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 µg/mL concentrations.

The extract and diluted standards was then read under UV-Visible Spectrophotometer Wagtech, CECIL 2021 at 450nm to obtain its optical density (OD) which was able to estimate the beta carotenes in the sample. Linear regression equation obtained from the standard plot and the beta carotenes content of the unknown calculated as described by (Rasaki et al., 2009).

2.7 Mineral Content (Zinc, Iron and Magnesium)

The analysis of minerals (zinc, iron and magnesium) were done according to AOAC [16] method number 968.08 procedures by the use of UNICAM, 919 Atomic Absorption Spectrophotometer (AAS). For each sample 5 g was measured in a pre-dried and weighed crucibles then incinerated at 550^{o}C overnight to ash. The ash was dissolved in 6 N HCl and left for 12hrs to allow extraction of minerals. The results were presented as an average of the duplicate determination.

2.8 Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 software statistical package. All values for chemical properties were presented as Mean ± SD. Statistical differences between extruded porridge samples were determined by Oneway Analysis of variance (ANOVA) and Post-hoc (Duncan Multiple Range Test) with a significance level of $p < 0.05$, so that significant differences between the created formulations to be observed.

3. RESULTS AND DISCUSSION

3.1 Nutritional Quality

Proximate composition results are shown in Table 2, and results of vitamin A, iron, zinc and magnesium content of the complementary porridge are shown in Table 3.

3.1.1 Moisture content

Moisture content of the extruded flour samples were significantly lower ($p < .05$) than of the control samples. This is assumed to be caused by the lower moisture content of the ingredient flours, including soybean (3%) flour. The high moisture content of the generated flour was caused by the high percentage of yellow maize (12%) in the control samples. Moisture content in the flour samples ranged from 3.40 g/100g in sample MPSC1 (55:15:20:10) to 4.74 g/100g in sample MS (75:25). However, all the formulated complementary porridges had a moisture content within the acceptable FAO/WHO requirement. The low moisture content might be due to drying and roasting of the sample raw materials during preparation process. Findings of this study agrees with a work reported by Chamba et al*.,* (2021) after going through the same procedures in the preparation of flour from yellow maize, soybeans and pumpkinseeds to reduce their moisture content. Given that spoilage microorganisms thrive in environments with high moisture content, therefore samples that have been formed with lower moisture content are going to have a longer shelf lives.

3.1.2 Carbohydrate content

Carbohydrate content in porridge samples increased with higher proportion of yellow maize (76.5%). Carbohydrate content in the flour samples ranged from 59.45 g/100g in sample MPSC1 (55:15:20:10) to 72.42 g/100g in sample MS (75:25). The complementary porridge samples can be regarded as good source of energy to support children's growth, this is because all of the flour samples had carbohydrate content within FAO/WHO requirements of 60-75. The findings of this study reflects results by Chamba et al., [6] that high carbohydrate content in developed composite flour increase with the utilization of yellow maize. Carbohydrates are an infant's main fuel source, and essential for proper growth and development. Offering your infant healthy, nutrient-dense carbohydrates will help optimize child growth and maintain a healthy body weight.

3.1.3 Protein content

Protein content of complementary porridge samples ranged from 18.45 g/100g in sample MPSC3 (65:20:10:5) to 14.08 g/100g in sample MP (75:25). Similarly, all of the formulated complementary flour samples had protein content within acceptable FAO/WHO requirement 15% as can be seen in the Table 2. except for a control sample MP (70:25). This is contributed by the utilization of protein rich ingredients (soybean (34%) and pumpkin seeds (30.3%) compared with the carbohydrate rich ingredients (maize) that were utilized in the formulation of the control samples. Since most cereals such as maize have a low protein content. The findings of this study are comparable to Marcel et al. [14] that high protein content in developed composite flour increases with the utilization of soybeans and pumpkin seeds.

"Cereal based complementary foods emanating from such ingredients should be augmented by protein rich (leguminous) foodstuffs to improve their nutritional significance" [18]. "The formulated complementary flour might be regarded as a good source of protein. Protein is vital for the prevention of protein–energy malnutrition (PEM), which is frequently witnessed among children in emerging nations, particularly during weaning" (Achidi et al., 2016; Adisetu et al., 2017). Protein makes important nutrient composition in complementary foods. They are major sources of essential amino acids and energy at times of energy deprivation. Adequate supply of dietary protein is vital for maintaining cellular function and integrity and for ensuring normalcy of health and growth.

3.1.4 Fibre content

Fibre is not digested by the body. It passes through your stomach, intestines, colon and then out of your body. Dietary fiber, often called roughage, is the indigestible plant-derived food component. Fibre plays a major role of increasing the utilization of nitrogen and absorption of some micronutrients (Lisanti, 2019). Fibre content in porridge samples ranged between 5.56 g/100g to 4.09 g/100g. FAO/WHO recommends complementary foods to contain low fibre as high fibre products can lead to high water absorption and displacement of nutrient and energy needed for the growth of children less than two years of age.

Daily recommended allowance of crude fiber in the complementary foods is <5% (FAO/WHO). "Also high-fiber foods effectively provide satiation by filling the stomach and delaying the absorption of nutrients. Such attributes of the complementary foods may also lower the child's feeding ability" [19].

3.1.5 Fat content

Crude fat contents of the extruded flour samples were significantly higher ($p < .05$) than the control sample. The results ranged from 11.89g/100g in sample MPSC1 (55:15:20:10) to 1.51g/g100 in sample MP (70:25:5). As shown in Table 2, this effect was contributed by the incorporation of pumpkin seeds (44.54% fat) and soybean (22.68% fat), which has good quantities of fats. On the contrary, the poor quantities of fats exhibited by the control samples are thought to be due to the utilization of carbohydrate rich ingredients including maize. Interestingly, all the formulated flour samples were within the FAO/WHO requirements of 10%–25%. These findings reflects results by Marcel et al. [14] that fat content in composite flour increase due to utilization of soybeans and pumpkinseeds. Fats content can affect developed food shelf life stability, this is because in the presence of oxygen fats can undergo rancidity which results in food spoilage. Therefore, flour sample with high fats content is more liable to the spoilage than the one with low fats content. Also fats constitute an important portion of nutrients obtained from foods. For infants and young children, they are source of energy, essential fatty acids, and fat soluble vitamins (A, D, E, and K). In addition, dietary fats have an important role in promoting good health and enhancing the sensory qualities of the foods.

3.1.6 Ash content

The crude ash content of the extruded flour samples were higher compared to that of the control samples (MS), but within the acceptable FAO/WHO requirement (<3%). Ash content signifies the presence of minerals in food samples (Laryea et al., 2018), and this denotes that the formulated complementary foods in this study are potential sources of minerals. Ash content in the formulated samples ranged from 2.70 g/100g in MPSC1 (55:15:20:10) to 2.20 g/100g in sample MS (75:25). The findings from this study are in line with the findings by Haque et al., (2013) that the proportion of soybean (at least 20%) resulted in high ash content of soybean flour as one of the ingredients, which could play a key role in mineral composition of the formulations.

3.1.7 Vitamin A

Vitamin A contents in formulated flour samples ranged from 324.5 µg/100g in MPSC1 (55:15:20:10) to 21 µg/100g in MP (75:25). The highest ratio of vitamin A content in formulated samples (MPSC1 and MPSC2) was due to the utilization of carrots which has high vitamin A contents 16706 µg compared to soybeans and pumpkin seeds. This effect was expected because the main reason of selecting carrots in this study was its contribution of vitamin A in a form of beta carotene. The findings from this study were in agreement with those of Tumwine and Atukwase (2018), who reported an increase in vitamin A after supplementing millet flour with carrot powder. However, the obtained value of sample MPSC3 (65:20:10:5), MP (75:25), MS (75:25) are below RDI (210-400), also below the RDA of vitamin A of (500µg and 300µg) for children aged 6-24 months (Lwelamira et al., 2013; Lutter, 2013; FAO [20]; Lutter and Dewey 2003). Vitamin A is essential for formation and maintenance of healthy skin, hair, and mucous membranes, proper vision and strengthening healthy immune and reproductive systems.

3.1.8 Zinc

Zinc results ranged from 22.58 mg/100g in sample MPSC2 (60:15:17:8) to 9.41 mg/100g in sample MS (75:25). Similarly, results show that all formulated flour samples are more than the DRI12 (3–8.40 mg/100 g) according to World Food Program (2018). “Therefore, the complementary porridges might be good sources of zinc because they can provide more than 50% of zinc DRI, which is considered sufficient. Thus, the porridges are considered suitable for use by the targeted groups which include children and also can be recommended to women of reproductive age. Similar to iron, zinc deficiency is also associated with stunting, anemia, and higher disease susceptibility” (Agbemafle et al., 2020; Bhutta et al., 2013).

Also zinc is a component of many enzymes in the body and is involved in most metabolic processes. Zinc plays a role in the following bodily functions such as formation of protein in the body and thus assists in wound healing, blood formation and taste perception.

Table 2. Proximate compositions (%dwb) of the formulated complementary porridge

Samples	Moisture	Crude protein	Crude fibre	Ash	Fat	Carbohydrate
MPSC1	3.40± 0.35	17.42± 0.01	5.14± 0.28	2.70± 0.03	11.89± 0.21	59.45± 0.56
MPSC2	3.59± 0.35	17.27± 0.04	5.25± 0.42	2.46± 0.01	9.04± 0.35	62.39± 0.42
MPSC3	3.86± 0.77	18.45± 0.01	5.38± 0.14	2.52± 0.02	4.41± 0.14	65.38± 0.35
MP	4.66± 0.98	14.08± 0.01	5.56± 0.49	2.31±0.03	3.83± 0.49	69.56± 0.14
MS	4.74±0.49	15.04± 0.03	4.09± 0.28	2.20± 0.01	1.51± 0.56	72.42± 0.21
FAO/WHO	<5	>15	<5	<3	10-25	60-75

Key: MPSC; composite mixture of yellow maize, pumpkin seeds, soy beans and carrots
MP (control); composite mixture of yellow maize and pumpkins
MS (control); composite mixture of yellow maize and soybeans

Table 3. Vitamin A and minerals content (%dwb) of the formulated complementary porridge

Samples	Vitamin A (µg)	Iron mg/100g	Zinc mg/100g	Magnesium mg/100g
MPSC1	324.5±60.10	13.22± 0.11	19.27 ±0.31	216.51± 0.62
MPSC2	310±5.65	14.50± 0.18	22.58± 0.55	268.34± 0.43
MPSC3	153± 0.00	13.53± 0.16	17.59± 0.23	281.33± 1.06
MP	21±4.24	45.54± 0.25	10.50± 0.70	31.08± 1.35
MS	103.5±7.77	17.08± 0.16	9.41± 0.39	15.49± 0.36
RDI	210-400	3.9-18.6	3-8.4	54-75

Key: MPSC; composite mixture of yellow maize, pumpkin seeds, soy beans and carrot flours
MP (control); mixture of yellow maize and pumpkins flours
MS (control); mixture of yellow maize and soybeans flours
Recommended dietary intake (RDI) source (WFP, 2018)

3.1.9 Magnesium

Magnesium amount in the formulated flour samples were significantly higher ($p < .05$) when compared to both the control samples and the DRI (54–75 mg/100 g). The results ranged from 281.33 mg/100 g in sample MPSC3 (65:20:10:5) to 31.08 mg/100 g in sample MS (75:25). Higher magnesium content of formulated samples is evidently due to the raw materials utilized in complementary food formulation especially utilization of (soybeans 289 mg and pumpkin seeds 592 mg). “Magnesium is crucial good for child’s health as it keeps the heart rhythm steady, strengthens the bones, supports a healthy immune system, and maintains normal muscle and nerve function” (Ndife et al*.,* 2020). Findings of this study corresponded with those of Chamba et al. [6], who reported an increase in magnesium content after supplementing yellow maize flour with soybeans and pumpkinseeds.

3.1.10 Iron

Iron content in the formulated flour samples ranged from 13.22 mg/100 g in sample MPSC1 (55:15:20:10) to 45.54 mg/100 g in sample MP (75:25). It is further noted that all formulated samples were able to meet RDI (3.9- 18.6 mg/100 g), therefore the formulated samples can be recommended as a good source of iron because it provides 50% of the iron RDI for feeding children. Iron is essential for all tissues in a young child’s developing body, blood formation and help to preserve the health of young children [21-28].

This mineral is a vital component of hemoglobin, the part of red blood cells that carries oxygen. The symptoms of iron deficiency include anemia, malabsorption of food, irritability, anorexia, pallor, and lethargy. Studies have also shown that iron deficiency in infants and older children may be associated with irreversible behavioral abnormalities and abnormal functioning of the brain.

4. CONCLUSION

Formulated flour samples MPSC1(55:15:20:10), MPSC2 (60:17:15:8) and MPSC3 (65:20:10:5) of this study were able to meet recommended daily intake for feeding children aged 6-24 month, compared to the reference samples (control). Sample MPSC3 is the best one as it contain high amount of protein and minerals and can be recommended for children diet to support their growth and health. Based on the targeted

nutrients of interest of this study, results showed that soybeans contributed to protein level, carrots contributed to high beta carotene amount, while pumpkin seeds contributed to zinc, magnesium and iron. This study proves that we can use extrusion to change lives by helping mothers to get nutritious foods to support the health of their growing babies, by producing baby foods using raw materials that are locally available in our markets. Also these foods can be used as a solution to children suffering from malnutrition and stop depending on commercial foods. These foods are highly nutritious and provide sufficient energy to support the children immunity, body growth and brain development.

COMPETING INTERESTS

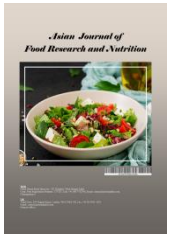
Authors have declared that no competing interests exist.

REFERENCES

1. Masuke R, Msuya SE, Mahande JM, Diarz, EJ, Stray-Pedersen B, Jahanpour O, Mgongo M. Effect of inappropriate complementary feeding practices on the nutritional status of children aged 6-24 months in urban Moshi, Northern Tanzania: Cohort study. PLoS One, 2021; 16(5):250-265.
2. Taghizade Moghaddam H, Khodaee GH, Ajilian Abbasi M, Saeidi M. Infant and young child feeding: a key area to improve child health. International Journal of Pediatrics. 2015;3(61):1083-1092.
3. Ogbo FA, Ogeleka P, Awosemo AO. Trends and determinants of complementary feeding practices in Tanzania, 2004–2016. Tropical Medical Health. 2018;46(40):18-20. Available:https://doi.org/10.1186/s41182-018-0121-x
4. Sen P, Mardinogulu A, Nielsen J. Selection of complementary foods based on optimal nutritional values. Scientific reports. 2017;7(1):1-9.
5. Abamecha N. Research review on formulation and sensory evaluation of complementary foods from cereals and legumes in Ethiopia. Food Science and Nutrition Technology. 2020;5(5):1-7.
6. Chamba G, Falmata AS, Bintu BP, Maryam BK, Modu S. Formulation and nutritional evaluation of high protein diet produced from yellow maize (*Zea Mays*) soya bean (*Glycine Max*), Pumpkin (*Cucurbita Pepo*) seed and fish (*Alestes Nurse*) meal. Open Journal of Bioscience Research (ISSN: 2734-2069). 2021;2(2): 36-65.
7. Choton S, Gupta N, Bandral JD, Anjum N, Choudary A. Extrusion technology and its application in food processing: A review. The Pharma Innovation Journal. 2020;*9*(2):162-168.
8. Rathod RP, Annapure US. Effect of extrusion process on antinutritional factors and protein and starch digestibility of lentil splits. LWT-Food Science and Technology. 2016;66(2):114-123.
9. Vivian Offiah, Vassilis Kontogiorgos, Kolawole O Falade. Extrusion processing of raw food materials and by-products: A review. Critical Review in Food Science and Nutrition. 2019;59(18):2979-2998. DOI:10.1080/10408398.2018.1480007
10. Harper JM, Clark JP. Food extrusion. Critical Reviews in Food Science and Nutrition. 2019;11(2):155-215.
11. Deenanath ED, Egal AA. Food extrusion technology: initiatives to address food and nutrition insecurity in South Africa. Journal of Pharmarcy and Nutrition Science. 2017;7(3):116-128.
12. Egal A, Oldewage Theron W. Extruded food products and their potential impact on food and nutrition security. South African Journal of Clinical Nutrition. 2020;*33*(4): 142-143.
13. Obinna Echem PC, Barber LI, Enyi CI. Proximate composition and sensory properties of complementary food formulated from malted pregelatinized maize, soybean and carrot flours. Journal of Food Research. 2018;7(2):17-24.
14. Marcel MR, Chacha JS, Ofoedu CE. Nutritional evaluation of complementary porridge formulated from orange fleshed sweet potato, amaranth grain, pumpkin seed, and soybean flours. Food Science & Nutrition. 2022;10(2):536-553.
15. Erhardt J. Nutrisurvey software version 2007; 2014.
16. AOAC. Official methods of analysis.16th Edition. Association of official analytical chemists. Washington DC, USA; 1999.
17. Rodriguez Amaya DB, Kimura M. *Harvest Plus handbook for carotenoid analysis*. Washington: International Food Policy Research Institute (IFPRI). 2004;2:63.
18. Adeniyi EA, Awotunde JB, Ogundokun R O, Kolawole PO, Abiodun MK, Adeniyi AA. Mobile health application and COVID-19:

Opportunities and challenges. Journal of Critical Reviews. 2020;7(15):3481-3488.
19. Rolfes SR, Pinna K, Whitney E. Understanding normal and clinical nutrition. Cengage learning; 2014.
20. FAO. Complementary Feeding for Children Aged 6-23 months. 2011;1–41. Available:http://www.fao.org/docrep/014/am866e/am866e00.PDF
21. Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. Journal of Food Nutrition. 2001;13(1):568–580.
22. Abeshu MA, Lelisa A, Geleta B. Complementary Feeding: Review of recommendations, feeding practices, and adequacy of homemade complementary food preparations in developing countries – lessons from Ethiopia. Frontiers in Nutrition. 2016;3(41):19. Available:https:/doi:10.3389/fnut.2016.00041 4110.3389/fnut.2016.00041
23. Black RE et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet.* 2013;382(9890): 427-513.
24. FAO/WHO. Protein Advisory Group (PAG) of the United Nations. Food and Agricultural Organization Rome. Guideline No 8. Protein–Rich Mixtures for Use as Weaning Food.New York: FAO/WHO/ UNICEF; 2017.
25. Laryea D, Wireko Manu FD, Oduro I. Formulation and characterization of sweetpotato-based complementary food. Cogent Food & Agriculture. 2018;4(1): 151-153.
26. PAHO / WHO. Guiding principles for complementary feeding of the breastfed child. Division of health promotion and protection. Food and nutrition program. Pan American Health Organization / World Health Organization; 2003
27. UNICEF. Tracking progress on child and maternal nutrition. A survival and development priority: New York; 2009.
28. World Health Organization. The world health report 2001: Complementary feeding: new understanding, new hope; 2001.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/98483



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 58-73, 2023; Article no.AJFRN.97723

Oxidative Stress Markers and Toxic Metals Assessment in Albino Wistar Rat fed with *Vigna unguiculata* Expose to Biopesticides (*Bacillus thuringiensis,* Neem *Azadirachta*) and Agrochemical

Oguh Collins Egwu [a*], Alexander Ikechukwu Ajai [b], Osuji Chigoziri Akudo [c], Ugwu Chukwuebuka Victor [d], Adinnu Chiamaka Maria-Goretti [e], Okeke Chioma Blessing [f], Ugwu Obiora Celestine [g], Obasi Glory Otuomasirichi [d], Umezinwa Ogochukwu Jennifer [h], Ugoeze Ucheoma Elele [i], Dickson Achimugu Musa [j] and Makun Husseini Anthony [k]

[a] Department of Subnatinal, Nigeria Center for Disease Control and Prevention, Abuja, Nigeria.
[b] Department of Chemistry, Federal University of Technology Minna, Niger State, Nigeria.
[c] Department of Biochemistry, Gregory University Uturu Abia State, Nigeria.
[d] Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.
[e] Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[f] Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria.
[g] Department of Pharmacology, Enugu State University of Science and Technology, Enugu State, Nigeria.
[h] Department of Science Laboratory Technology, University of Nigeria, Nsukka, Enugu State, Nigeria.
[i] Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.
[j] Department of Biohemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.
[k] Department of Biochemistry, Federal University of Technology Minna, Niger State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OCE involved in the planting, and the writing of the manuscript. Authors AIA and OCA contributed to the manuscript. Authors UCV and ACM contributed in the literature review. Author OCB involve in the sampling and preparation of reagent. Authors UOC involve in the metal analysis. Authors OGO involve in the statistical analyses. Authors UOJ involves in the estimations and calculations. Authors UUE and MHA read and edit the article before publication. All authors read and approved the final manuscript.

**Corresponding author: E-mail: collinsoguh@gmail.com;*

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97723

Original Research Article

Received: 19/01/2023
Accepted: 23/03/2023
Published: 24/04/2023

ABSTRACT

The study aimed to determine the Oxidative Stress markers and Toxic metals risk assessment in Albino Wistar rat fed with *Vigna unguiculata* expose to biopesticides *Bacillus thuringiensis* (*Bt*), Neem seed oil *Azadirachta* (*Aza*) and agrochemical (Lambda_cyhalothrin 15g/l and Dimethoate 300g/l) use for pest control. Dried mature *Vigna unguiculata* seed was randomly collected from four different field locations where Biopesticide (*Bt,* and *Aza*), agrochemical, and control were used to manage pest. Standard procedures were used to determine the physicochemical parameters of the soil samples and oxidative stress on the Albino Wistar rat fed. Phytate and oxalate contents were evaluated using the titrimetric method, while cyanogenic glycoside, tannin, and alkaloid concentrations were determined using the Pearson method. Atomic Absorption Spectrophotometry was used to determine the concentrations of toxic metals. Standard formulas were used to estimate the health risk assessment. The results shows that agrochemical led to a significant ($P< 0.05$) increased in lipid peroxidation in the rat blood sample, antinutrient factors, heavy metals and a significant decrease in the activities of the antioxidants enzymes: Superoxide dismutase, catalase and xanthine oxidase activities in the blood and cowpea seed compared to the biopesticides. Heavy metal contamination in seeds of cowpea controlled with agrochemicals had a hazard quotient and Hazard Index greater than 1, which indicates unsafety especially to children. The study concludes that biopesticides such as *Bt* and *Aza* have shown to be an alternative method in cowpea pest control with very less effect.

Keywords: Agrochemical; antinutrient; biopesticides; heavy metal; oxidative stress; risk assessment.

ABBREVIATIONS

LcD: Lambda_cyhalothrin 15g/l and Dimethoate 300g/l; BAF: Bioaccumulation factors; DIM: Daily intake of metal; ADDM: Average daily dose of metal; MC: Metal concentration; BW: Body weight; HQ: Hazard quotient; RFD: Reference oral dose; HI: Hazard index; CRD: completely randomized design; LP: Lipid peroxidation; SOD: superoxide dismutase; XO: xanthine oxidase; CAT: catalase; BHT: Butylated hydroxyl toluene; Bt: Bacillus thuringiensis; Aza: Azadirachta.

1. INTRODUCTION

Pests damage cause considerable crop losses and yield in recent years. To avoid these losses, farmers are now increasingly employing agropesticides in their agronomic practices to prevent losses and low yield. Pesticides are chemical substances that are used in agriculture to repel, prevent, and eradicate pests in order to increase yield. Agrochemicals have significantly increased agricultural productivity, but residual concentrations in the soil and potential ecosystem dangers are big concerns. Insects have a significant impact on African cowpea crop yields, influencing leaves, flower and stem component and also stage of development. The legume pod borer, Maruca vitrata, is the main preharvest pest of cowpeas [1]. The legume bug cause damage at all stages of development, more harm occurs during flowering [2]. Pesticides used to control these pests and prevent harm, especially those made of synthetic materials, and have a number of negative effects on humans and the environment [3].

An imbalance between free radicals and antioxidants in the body causes oxidative stress. Free radicals are oxygen-containing molecules with an uneven number of electrons. They can easily interact with other molecules because of their unequal quantity. Lipid peroxidation (LP) precedes oxidative damage in plants and animals. Antioxidant defense mechanisms, on the other hand, are found in living organism. Antioxidant defense mechanisms include enzymes like superoxide dismutase (SOD), xanthine oxidase (XO) and catalase (CAT), as well as non-enzymes like ascorbic acid. Oxidative stress is measured by changes in the levels of these antioxidants. Furthermore, the activity of xanthine oxidase is a measure of oxidative stress as well as a defense mechanism [4].

Long-term use of synthetic pesticides in agriculture has resulted in the accumulation of pesticidal residues in the environment as a result of run-off, and also heavy metals which are not biodegradable, has led to a variety of chronic illnesses and non-target organism toxicity. Heavy metals also transported to humans through the food chain, where they may cause variety of human health issues [5, 6]. Synthetic pesticides have shown to be effective in pest management but increasing focus is being placed on the creation of ecologically friendly pesticides that will aid in the efficient management of pests while also reducing chronic health issues [7]. One of the most important alternative strategies is the use of Biopesticides (*Bt* and *Aza*) [8].

Bacillus thuringiensis (Bt) has been employed in agriculture because of its insecticidal proteins, making it an environmentally friendly biopesticide. The presence of -endotoxins, particularly cry protein, is what gives the bacteria its insecticidal properties. Its application, however, is not limited to only insecticidal property but also a biofertilizer for boosting plant growth, the generation of transgenic plants, and other applications has been demonstrated in previous studies [9, 10]. Neem oil has parasitic, insecticidal spermicidal properties, killing a wide variety of organisms, including pests [11]. Neem's constituent phytochemicals have been discovered to have a wide range of therapeutic benefits [12]. Azadirachtin is the most active complex secondary metabolite identified in neem seeds, which has long been known as an important insecticidal component. In insects, it acts as an antifeedant, and repellant that is Neem prevent insects from feeding [13, 14].

Anti-nutritional factors are compounds present in food that interfere with beneficial nutrients, minerals, and metabolic processes from being absorbed, as well as reducing the bioavailability of nutrients from plants or plant products used as human diets. When antinutrients such as cynogenic glycoside and alkaloid are consumed in high concentrations, they hinder cells from utilising oxygen, which can lead to infertility, cancer, gastrointestinal and neurological disorders [15, 16]. Phytate, oxalate, and tannins reduce the bioavailability of proteins, carbohydrates, and essential minerals like calcium, magnesium, zinc, iron, and phosphorus by forming insoluble complexes that aren't easily absorbed by the gastrointestinal tract, resulting in health problems like oxalemia [17 - 20].

Cowpea are mostly damage by insects, so synthetic chemicals is regularly use to control pest and for the millions of people that consume them, this is a huge health risk. Agrochemicals are quick and easy way to eliminate pests in the field which increases long-term toxicity risks to people and other ecosystem biota. Nonetheless, biopesticides properties have been discovered in neem plant and in *Bt* as an alternative to agrochemicals. Hence, this study aimed to determine the Oxidative Stress markers and Toxic metals risk assessment in Albino Wistar rat fed with *Vigna unguiculata* expose to biopesticides *Bacillus thuringiensis* (*Bt*), Neem seed oil *Azadirachta indica* (*Aza*) and agrochemical (Lambda_cyhalothrin 15g/l and Dimethoate 300g/l) use for pest control.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Biochemistry Department Federal University of Technology Minna Niger State, Bosso Campus. Bosso is situated at 9º65’ North latitude, 6º52’ East longitude, with an area of 72km^2.

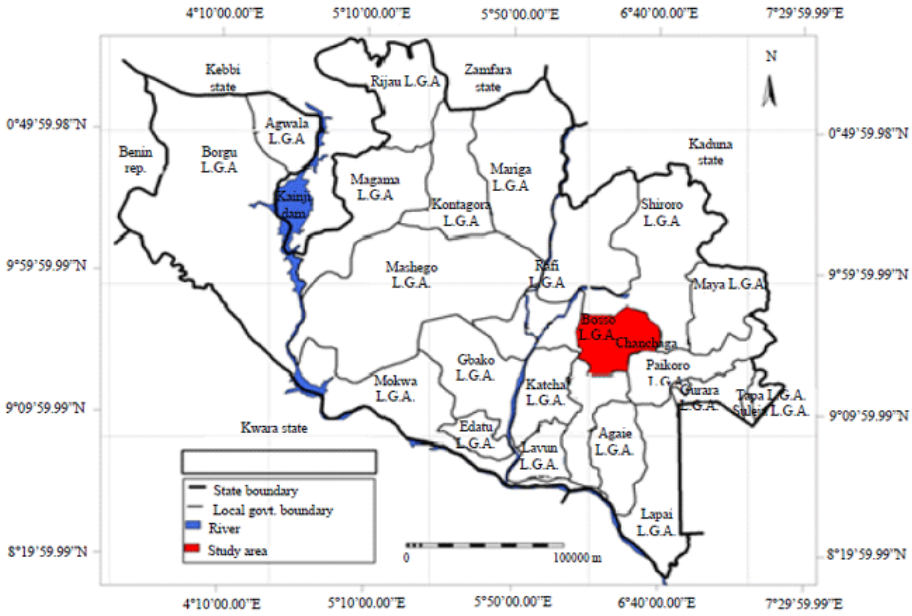


Fig. 1. Map of Niger State Showing study area in red spot

2.2 Collection of Experimental Rats, Soil and Biopesticides

Top soil sample about seven hundred grams use for planting was collected 6 inches (15cm) below the surface with a sterile hand shovel from a fallow land from Agriculture department Federal University of Technology, Minna and was used to test different soil physicochemical properties. Biopesticide *Bt* and neem oil was purchase from Konga online and the instruction for use were strictly followed. Twelve mature experimental male AlbinoWistar rats weighing 192 -200g were purchase from animal house Federal University of Technology Minna.

2.3 Experimental Design and Planting of Seed

The soil was divided into four groups and each group had twenty seven perforated polythene bags with 2kg of soil. Three seeds were planted in each test bag to an approximate depth of 2 cm. Biopesticides (*Bt* and *Aza*), agrochemical, and a control without treatment were used in groups A, B, C and D respectively. Biopesticides and Agrochemical were sprayed on the test crops from seven weeks of planting when blooming and flowering began to suppress insects, except on the control groups. And the number of spot on cowpea pods damage by pest was observed by circling the damaged spot with a permanent marker. To avoid counting the same spot twice, the counted spots were marked.

Twelve mature male AlbinoWistar rats weighing 192 -200g were randomly assigned into four groups of three rats each. Groups 1, 2, 3 and 4 were fed with cowpea treated with Biopesticides (*Bt* and *Aza*), agrochemical and control for 12 weeks.

2.4 Sample Collection

Dried Seeds of cowpea from each group and their corresponding soil were randomly collected for analysis from each group were biopesticides (*Bt* and *Aza*), agrochemical was applied for pest control and each samples from four different location where use to feed rats. Animal blood was used to determine the level of oxidative stress. The soil used for planting was mixed, then air-dried for seven days at room temperature (27^{o}C) to stop all microbial activity in the soil. Using 2mm sieve mesh size, the air-dried soil samples were sieved and handpicked to remove trash and stones and was used to test different soil physicochemical properties before and after harvest.

2.5 Determination of the Physico-Chemical Parameters

A potentiometric meter and a digital pH meter were used to determine the pH of the soil samples. About 10 g of soil samples with 100 ml of distilled water using a glass rod to agitate, and pH of the suspension was determined. The physicochemical parameters of the soil were

examined before and after treatment using [21]. The physicochemical variables tested were soil texture, pH, total organic carbon, organic matter, total nitrogen, total phosphorus, and exchangeable cation (sodium ion, magnessium and potassium ion) to determine the pesticide's biodegradability.

2.6 Preparation of Extracts for the Determination of Oxidative Stress Markers in Cowpea Seed

Three drops of butylated hydroxyl toluene (BHT) and 0.05 M phosphate buffer pH 7.5 were added to blood sample, and centrifuged at 5000 g for 10 min. The supernatant was used to determine oxidative stress indicators.

2.6.1 Determination of lipid peroxidation markers in cowpea seedlings

The assay is based on the reaction of malondialdehyde (MDA) with thiobabituric acid (TBA); forming a MDA-TBA2 adduct that absorbs strongly at 532 nm. Acetic acid (1.0 ml) was placed in a test tube and 1.0 ml of 10% TBA was added to the tube followed by 0.1 ml of the blood supernatant. The test tube was covered and immersed in boiling water for 15 min. After cooling the mixture, it was centrifuged at 5000 g for 10 minutes. The spectrophotometer was zeroed and absorbance of test sample was read at 532 nm against the reagent blank [4].

2.6.2 Determination of superoxide dismutase activity in cowpea seed

The process inhibits auto-oxidation of adrenaline from turning into adrenochrome. About 2.5 ml of a 0.05 M phosphate buffer with a pH of 7.4 were added to 2 ml of the homogenate. 0.5 ml of freshly made 0.3 nm epinephrine was added to the buffer-supernatant mixture to initiate the reaction. This was mixed by inversion.

Exact 2.5 ml of the buffer, 0.5 ml of epinephrine, and 2 ml of deionized water were contained in the reference cuvette. The rise in absorbance at 480 nm was monitored every second for 150 second. The amount of enzyme necessary to inhibit epinephrine's oxidation to adrenochrome by 50% at a rate of 480 nm per minute is known as one unit of superoxide dismutase activity [22, 23]. An Sp 1800 UV/VIS Spectrophptometer was used to assay the enzyme activity.

2.6.3 Determination of catalase activity in cowpea seedlings

Hydrogen peroxide is broken down by catalase to produce oxygen, which oxidizes potassium dichromate. A chromophore with a maximum absorption at 610 nm results from the oxidation of chromate. The reaction mixture contained 1 ml of 0.05 M phosphate buffer (pH 7.5), 0.5 ml of 0.2 M H_2O_2, and 0.4 ml H_2O. The enzyme extract (0.5 ml) was added to the reaction mixture, and the mixture was then incubated for different time periods, t1, t2, and t3, for 1 minute, 2 minutes, and 3 minutes, respectively. After each interval, the reaction was stopped by adding 2 ml of the acid reagent (dichromate/acetic acid mixture), which was made by combining glacial acetic acid and potassium dichromate at a 5% concentration (1:3 by volume). The enzyme was added to the control following the acid reagent. The absorbance was measured at 610 nm with a Sp 1800 UV/VIS Spectrophptometer after all the tubes had been boiled in boiling water for 10 minutes. Catalase activity was measured in moles of H_2O_2 used per minute [24].

2.6.4 Determination of xanthine oxidase (XO) activities in cowpea seedlings

Xanthine oxidase is an enzyme that catalyses the conversion of methylene blue to the reduced colorless forms. The reciprocal of the amount of time it takes for methylene blue to turn colorless is used to measure enzyme activity. A test tube rack was filled with two test tubes labeled "control" and "test," and one milliliter of neutral formaldehyde solution at 0.05% was pipetted into each test tube. In the test tube marked "test," the 0.02% methylene blue solution was added. Next, 1 ml of the blood supernatant was added to the corresponding test tube. 1 ml of distilled water was added to the control test tube, and in order to prevent air oxidation, 2 drops of liquid paraffin were also added to the both test tube [4].

2.7 Anti-nutrient Analysis

Titrimetric method of Association of official analytical chemist AOAC, [25], was used to estimate oxalate and phytate content while [26] method was use to estimate cyanogenic glycoside, tannin and Alkaloid content.

2.8 Determination of Heavy Metal

Blood, Soil and cowpea seed samples (1ml: 1.00:0.1g each) were placed in separate 100ml

beakers and given 15ml of a tri-acid mixture (70 percent high purity HNO_3, 65% $HClO_4$, and 70 percent H_2S0_4 in a 5:1:1 ratio). The solution was digested at 800°C till it became transparent. The resultant solution was filtered and dilute to 50mL with deionized water before being examined using atomic absorption spectrophotometry for As, Pb, Cr, Cd, Cu, and Hg [27].

2.9 Assessment of Human Health Risk

2.9.1 Bioaccumulation factor (BAF) estimation

The transfer coefficient (transfer or metal uptake from soil via cowpea seed) was calculated using [28].

$$BAF = C_{seed}/C_{soil} \quad (1)$$

C_{seed} = metal concentration in cowpea seed, mg/kg
C_{soil} = milligrams of metal per kilogram of dry weight of soil.
BAF greater than 1 signifies that the cowpea enriched metal from the soil.
BAF less than 1 indicates that the cowpea exclude metals from the soil

2.9.2 Estimation of the daily intake of metal (DIM)

The following formula was used to calculate the daily metal intake [29].

$$ADDM = DI \times MC_{seed}/BW \quad (2)$$

Where;

ADDM = indicates average daily dose of metal (mg,kg/d).
DI = Cowpea seed daily intake (0.83 kg/d for adults, 0.88 kg/d for children).
MC_{seed} = is the metal concentration in the seed (mg/kg)
BW = Indicate the body weight of average individual 55.7kg for adults and 14.2kg for Children).

2.9.3 Estimation of hazard quotient HQ

The Hazard Quotient (HQ) assess the possible risks to human health associated with consumption of these cowpea grown in pesticide-contaminated soil using the following equation [30].

HQ is the ratio between exposure and the reference oral dose (RFD)

Ratio lower than one 1, means no obvious risk.

$$HQ = ADDM/RFDM \quad (3)$$

Where;

ADDM = The average daily dose (mg,kg/d) of the metal
RFDM = The reference dose of metal (mg,kg/d) which is the maximum tolerable daily intake of metal with no adverse effect

2.9.4 Estimation of Hazard Index (HI)

The HI assess the total risk of heavy metal exposure from consuming a particular cowpea [31]. The value of the hazard index is proportional to the level of toxicity in the cowpea consumed. If the HI value is more than one, the anticipated exposure is likely to cause health problems.

$$HI = \sum HQ_{As} + HQ_{Cu} + HQ_{Pb} + HQ_{Cd} + HQ_{Cr} + HQ_{Hg} \quad (4)$$

2.9.5 Analytical statistics

The data was analyzed using IBM Statistical Product and Service Solution (SPSS) version 20 and Microsoft Excel 2013. The information was presented in the form of a mean and standard deviation (SD). One-way analysis of variance (ANOVA) was use for significant different. Duncan's multiple range test (DMRT) was used to compare mean values across test groups and controls, as well as between test group means.

3. RESULTS

3.1 Physicochemical Properties of Soil Samples before and After Planting

Table 1 summarizes the physicochemical properties of soil samples. The pH of the soil was 6.91, 6.51, 6.42, 4.25 and 6.57 before planting (control soil with no pesticide), after planting (control soil with no pesticide), soil with *Aza* solution, soil with synthetic agrochemical, and *Bt* soil respectively.

3.2 Physical Observation on the Number of Spot on *Vigna unguiculata* Pod Damage by Pest

Table 2 shows the total number of injured pods in the test samples and the control. The

observations continued for another five weeks after the seventh week of planting. Pod of cowpea was randomly peak from each field and the group that got no treatment had the maximum damage on the pods, with 55 places of damage, while *Vigna u* treated with *Aza, Bt* and agrochemical had 13, 10 and 9 spots of damage, respectively.

Table 1. Physicochemical properties of soil samples before and after planting

Soil properties	(Before Planting)	Control soil*	*Aza* soil*	Agro soil*	*Bt* soil*
Texture	**loamy**	**loamy**	**loamy**	**loamy**	**loamy**
pH	6.91 ± 0.03^{a}	6.51 ± 0.03^{b}	6.42 ± 0.03^{c}	4.25 ± 0.03^{d}	6.57 ± 0.03^{b}
Total N %	1.96 ± 0.04^{a}	1.92 ± 0.03^{a}	1.88 ± 0.03^{b}	1.52 ± 0.06^{c}	1.95 ± 0.03^{a}
Total P%	20.84 ± 0.19^{a}	20.78 ± 0.1^{a}	18.69 ± 0.07^{b}	18.47 ± 0.15^{c}	18.56 ± 0.07^{b}
OM %	3.78 ± 0.10^{a}	3.82 ± 0.01^{a}	3.66 ± 0.11^{a}	3.50 ± 0.06^{b}	3.73 ± 0.11^{a}
OC%	2.67 ± 0.06^{b}	2.64 ± 0.05^{b}	2.28 ± 0.03^{c}	2.75 ± 0.04^{a}	2.18 ± 0.03^{d}
K^{+} meq/100g	1.99 ± 0.03^{a}	1.97 ± 0.04^{a}	1.76 ± 0.04^{b}	1.64 ± 0.04^{c}	1.52 ± 0.04^{c}
Mg^{2+} meq/100g	13.25 ± 0.02^{a}	13.21 ± 0.03^{a}	12.29 ± 0.08^{b}	11.50 ± 0.04^{c}	12.17 ± 0.08^{b}
Na^{+} meq/100g	8.16 ± 0.06^{a}	8.13 ± 0.04^{a}	7.98 ± 0.03^{b}	7.86 ± 0.06^{c}	8.05 ± 0.03^{b}

*Results expressed as Mean ± SD. Mean values with same superscript letters on the rows are considered not significant (P>0.05). n=3 ** = After planting*

Table 2. Physical observation on the number of spot on *Vigna u.* damage by pest

Weeks	Control	*Vigna u.* with *Aza*	*Vigna u* with *Bt*	*Vigna u.* with Agro
7	15	5	3	4
8	13	4	2	3
9	10	2	2	1
10	9	1	2	1
11	8	1	1	0
Total spot	55	13	10	9

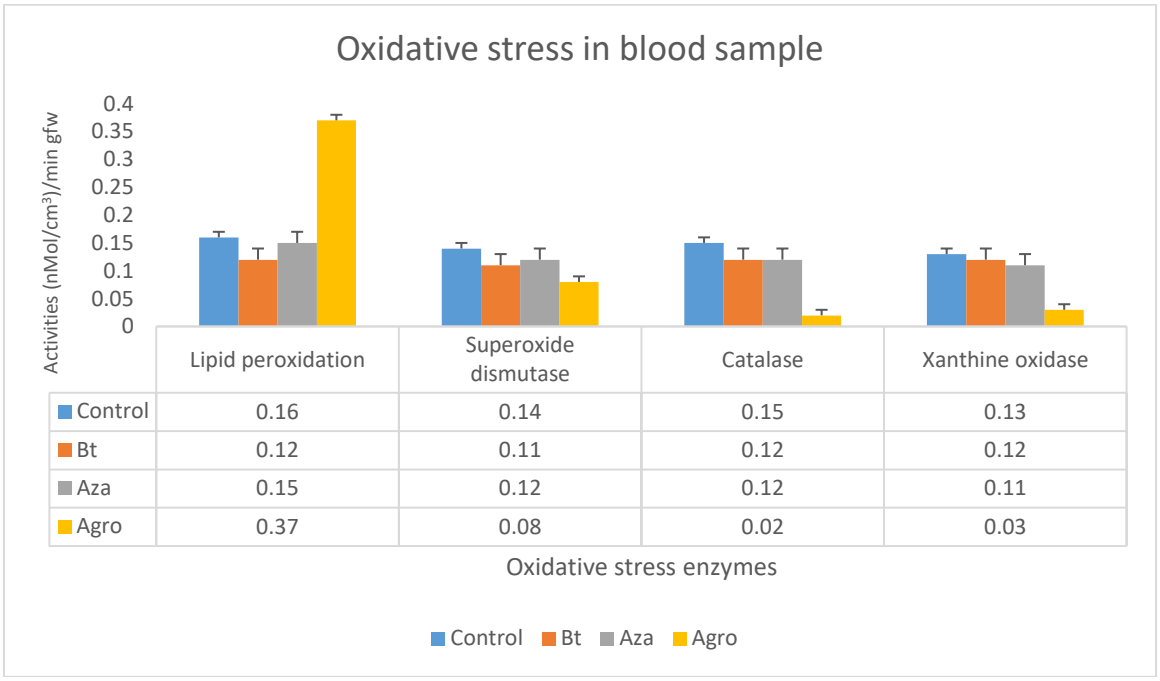


Fig. 2. Oxidative stress enzymes in blood

3.3 Oxidative Stress Markers in Blood Sample

Lipid peroxidation and antioxidant enzymes; superoxide dismutase, catalase and xanthine oxidase are shown in Fig. 2. Lipid peroxidation in rat blood fed with agro pesticides cowpea was found to increase significantly when compared with the control ($p < 0.05$). The value of LP in the control, *Bt, Aza* and agro were 0.16, 0.12, 0.15 and 0.37 respectively. The activities of SOD, CAT, and XO significant decrease ($p < 0.05$) in the blood of rat fed with cowpea grown with *Bt, Aza*, and Agro compared with the control.

3.4 Antinutrient Factors in *Vigna unguiculata*

The cyanogenic and oxalate levels from Agro chemical are 2.56 mg/100g and 25.32 mg/g^{-1} and are within the limits in cowpea 0.5 - 3.5 mg/kg and 500 mg/100g respectively. Phytate (12.39), Alkaloid (1.37) and tannin (1.46) contents in Cowpea seeds cultivated with agrochemical were above the threshold in cowpea 0.035 %, 0.02 % and 0.25 g/l respectively.

3.5 Heavy Metal Concentration in Soils

Heavy metals As, Pb, Cu, Cd, Cr, and Hg concentrations in soil with Aza, Bt, agrochemical, and no pesticide were (2.08, 2.54, 2.81, 0.52, 3.55, 0.38), (2.10, 2.58, 2.89, 0.75, 3.87, 0.52), (5.78, 8.89, 4.52, 3.74, 8.46, 4.48), and (2.03, 2.35, 0.72, 0.39, 3.46, 0.36 mg/kg), respectively. According to the study, the concentrations of the heavy were significantly ($p<0.05$) higher in the soil with agrochemical pesticide than other tested samples (Table 4). In the soil with the *Aza and Bt*, the majority of the metals analyzed were identified in the lowest quantities.

3.6 Heavy Metal Level in *Vigna u* Controlled with Neem, Bt and Agro Pesticide

Vigna u with *Bt, Aza*, agrochemical, and control without pesticide mean levels of heavy metals are summarized in Table 5. In *Vigna u*, the concentrations of As, Pb, Cu, Cd, Cr, and Hg in *Bt* (0.12, 0.14, 0.91, 0.03, 0.08, 0.18), *Aza* (0.32, 0.57, 2.78, 0.09, 0.30, 0.27), agrochemical (4.06, 7.41, 3.53, 2.04, 4.86, 3.04), and without pesticide (0.02, 0.51, 1.33, 0.21, 0.29, 0.17 mg/kg). All metal levels in *Vigna u* with agrochemicals were greater than the FAO/WHO limit of metal in cowpea 0.5, 2.0, 0.04, 0.5, 0.3, and 0.1 mg/kg for As, Pb, Cu, Cd, Cr, and Hg, respectively. The metal Cu and Hg contents in cowpeas with Agro chemical were more (2.78 and 0.27 mg/kg, respectively), which exceeds the permissible limits (0.04 and 0.1 mg/kg).

Table 3. Antinutrient factors in cowpea controlled with agrochemical and neem solution

Antinutrient factors (mg/100g)	Agro	Cowpea samples		Control	Limit	Source
		Aza	*Bt*			
Cyanogenic	2.56 ± 0.03^{a}	0.56 ± 0.01^{b}	0.54 ± 0.01^{b}	0.23 ± 0.08^{c}	0.5 – 3.5	[32]
Phytate(g/100g)	12.39 ± 0.23^{a}	4.12 ± 0.23^{b}	4.18 ± 0.23^{b}	2.4 ± 0.03^{c}	0.035 %	[33]
Oxalate (mg/g^{-1})	25.32 ± 0.81^{a}	9.29 ± 0.15^{b}	9.32 ± 0.15^{b}	5.71 ± 0.13^{c}	200–500	[34]
Alkaloid %	1.37 ± 0.01^{a}	0.02 ± 0.01^{b}	0.01 ± 0.01^{c}	0.02 ± 0.01^{b}	0.02 %	[35]
Tannin g/l	1.46 ± 0.04^{a}	0.19 ± 0.03^{b}	0.20 ± 0.03^{b}	0.12 ± 0.05^{c}	0.25 g/l	[36]

Table 4. Heavy metal concentration in soils with *Aza, Bt* and Agro pesticide

Heavy metals (mg/kg)	Samples				
	Aza	*Bt*	Agro	Control	PL(mg/kg) in soil FAO/WHO [37, 38]
As	2.08 ± 0.04^{b}	2.10 ± 0.04^{b}	5.78 ± 0.12^{a}	2.03 ± 0.03^{b}	20
Pb	2.54 ± 0.10^{c}	2.58 ± 0.10^{c}	8.89 ± 0.04^{a}	2.35 ± 0.13^{b}	50
Cu	2.81 ± 0.09^{b}	2.89 ± 0.09^{b}	4.52 ± 0.10^{a}	0.72 ± 0.18^{b}	100
Cd	0.52 ± 0.06^{c}	0.75 ± 0.06^{b}	3.74 ± 0.04^{a}	0.39 ± 0.03^{d}	3.0
Cr	3.55 ± 0.03^{c}	3.87 ± 0.03^{b}	8.46 ± 0.07^{a}	3.46 ± 0.37^{c}	100
Hg	0.38 ± 0.07^{c}	0.52 ± 0.07^{b}	4.48 ± 0.04^{a}	0.36 ± 0.09^{c}	2.0

Mean values with same superscript letters on the rows are considered not significant (P>0.05). PL= Permissible limit

Table 5. Heavy Metal Concentration in *Vigna u.* treated with *Bt, Aza* and agrochemical pesticide

Heavy metals (mg/kg)	Samples				
	Vigna u with *Bt*	*Vigna u* with *Aza*	*Vigna u* with Agro	Control pesticide	PL (mg/kg) in *Vigna u* FAO/WHO, [39*,40]**
As	0.12 ± 0.04^{c}	0.32 ± 0.04^{b}	4.06 ± 0.04^{a}	0.02 ± 0.02^{d}	0.5*
Pb	0.14 ± 0.04^{c}	0.57 ± 0.04^{b}	7.41 ± 0.02^{a}	0.51 ± 0.13^{b}	2.0*
Cu	0.91 ± 0.03^{c}	2.78 ± 0.03^{b}	3.78 ± 0.11^{a}	0.33 ± 0.02^{c}	0.04**
Cd	0.03 ± 0.01^{c}	0.09 ± 0.01^{b}	2.04 ± 0.02^{a}	0.21 ± 0.01^{c}	0.5*
Cr	0.08 ± 0.05^{c}	0.30 ± 0.05^{b}	4.86 ± 0.03^{a}	0.29 ± 0.02^{b}	0.3*
Hg	0.18 ± 0.01^{b}	0.27 ± 0.01^{b}	3.04 ± 0.02^{a}	0.17 ± 0.01^{b}	0.1*

Mean values with same superscript letters on the rows are considered not significant (P>0.05)

Table 6. Heavy Metal level in blood of rat fed with *Vigna u*

Heavy metals (µg/L)	Samples				
	Vigna u with *Bt*	*Vigna u* with *Aza*	*Vigna u* with Agro	Control pesticide	Limit (µg/L) in blood metal
As	0.49 ± 0.08^{c}	1.34 ± 0.05^{b}	6.72 ± 0.07^{a}	0.45 ± 0.07^{c}	3.12 [41]
Pb	0.94 ± 0.02^{b}	0.97 ± 0.03^{b}	9.41 ± 0.09^{a}	0.34 ± 0.10^{c}	2.0 [42]
Cu	2.73 ± 0.06^{c}	3.65 ± 0.07^{b}	3.82 ± 0.92^{a}	0.67 ± 0.07^{c}	1495 [41]
Cd	0.08 ± 0.05^{c}	0.10 ± 0.08^{b}	1.21 ± 0.08^{a}	0.06 ± 0.01^{c}	0.15 [42]
Cr	0.04 ± 0.05^{c}	0.22 ± 0.05^{b}	7.95 ± 1.10^{a}	0.31 ± 0.06^{b}	1.86 [41]
Hg	0.06 ± 0.02^{b}	0.09 ± 0.02^{b}	2.07 ± 0.02^{a}	0.08 ± 0.01^{b}	0.1 [39]

Mean values with same superscript letters on the rows are considered not significant (P>0.05)

Table 7. Estimation of bioaccumulation factor (BAF)

Heavy metals (mg/kg)	BAF			
	Bt	Aza	Agrochemical	Control
As	0.06	0.15	0.70	0.01
Pb	0.05	0.22	0.83	0.21
Cu	0.31	0.98	0.83	0.45
Cd	0.04	0.17	0.54	0.53
Cr	0.02	0.08	0.57	0.08
Hg	0.35	0.71	0.67	0.47

3.7 Heavy Metal Level in Rat Blood Samples fed with *Vigna u* with Neem, Bt and agro Pesticide

Blood sample of rat fed with expose *Vigna u* with *Bt, Aza*, agrochemical, and control mean levels of heavy metals are summarized in Table 6. All metal levels in blood sample of *Vigna u* with agrochemicals were greater than the limit of metal in blood 3.12, 2.0, 1495, 0.15, 1.86, and 0.1 µg/L for As, Pb, Cu, Cd, Cr, and Hg, respectively.

3.8 Estimation of Bioaccumulation Factor (BAF)

Shows the bioaccumulation factor (BAF) of heavy metals from soil to cowpea plants, which is the ratio of metal concentration in cowpea to total soil concentration. In *vigna u* treated with *Bt, Aza*, agrochemical, and no pesticide, the bioaccumulation factors of metals As, Pb, Cu, Cd, Cr, and Hg were (0.06, 0.05, 0.31, 0.04, 0.02 and 0.35), (0.15, 0.22, 0.98, 0.17, 0.08 and 0.71), (0.70, 0.83, 0.83, 0.54, 0.57 and 0.67), and (0.01, 0.21, 0.45, 0.53, 0.08 and 0.47), respectively.

3.9 Daily Intake and Potential Hazard (Hazard Quotient) of Metal in Human

Daily intake and hazard quotient that will be derived from trace metal consumption in *Vigna u* for both adults and children are shown in Table 8. The estimated daily intake of heavy metals (DIM) was calculated using the average cowpea consumption for both adults and children. The HQ of heavy metal detect a significant quantity of Cu in adults (1.04 and 1.41) and children (4.31 and 5.86) in *Vigna u* treated with *Aza* and agropesticide (4.31 and 5.86). A high amount of Cr (1.00) and Hg (2.78) HQ was found in *Vigna u* with agropesticide for children.

Table 8. Daily Intake and Potential Hazard (Hazard Quotient) of metal in human

Heavy metals	DIM and HQ for individuals					
	Individuals	Hazards	*Vigna u* with *Bt*	*Vigna u* with *Aza*	*Vigna u* with agrochemical	Control
As	Adult	DIM	0.00	0.00	0.06	0.00
		HQ	0.00	0.01	0.12	0.00
	Children	DIM	0.00	0.02	0.25	0.00
		HQ	0.01	0.04	0.50	0.00
Pb	Adult	DIM	0.00	0.01	0.11	0.01
		HQ	0.00	0.00	0.06	0.00
	Children	DIM	0.00	0.04	0.46	0.03
		HQ	0.00	0.02	0.23	0.02
Cu	Adult	DIM	0.01	0.04	0.06	0.00
		HQ	0.33	1.04	1.41	0.12
	Children	DIM	0.01	0.17	0.23	0.02
		HQ	0.29	4.31	5.86	0.51
Cd	Adult	DIM	0.00	0.00	0.03	0.00
		HQ	0.00	0.00	0.06	0.01
	Children	DIM	0.00	0.01	0.13	0.01
		HQ	0.00	0.01	0.25	0.03
Cr	Adult	DIM	0.00	0.00	0.07	0.00
		HQ	0.00	0.01	0.24	0.01
	Children	DIM	0.00	0.02	0.30	0.02
		HQ	0.02	0.06	1.00	0.06
Hg	Adult	DIM	0.00	0.00	0.05	0.00
		HQ	0.03	0.04	0.45	0.03
	Children	DIM	0.01	0.02	0.23	0.01
		HQ	0.11	0.17	2.78	0.10

DIM = Daily intake of metal, HQ = Hazard quotient

Table 9. Estimation of hazard index (HI) of metal for adult and children

	HI for Individuals				
	Individuals	*Bt*	*Aza*	Agrochemical	Control
HI=∑HQ (HM)	Adult	0.36	1.1	2.34	0.17
	Children	0.44	4.61	10.62	0.72

HI = Hazard index. ∑ = Summation of the Hazard Quotient (HQ) arising from all the heavy metals (HM) examined

3.9.1 Estimation of hazard index (HI) of metal for individuals

Adult and children HIs in *vigna u* controlled with *Aza*, and agrochemical were all greater than 1, indicating toxicity, especially for the agrochemical pesticide, which had a 10.62 HI for children. *Bt* value is below 1, which indicate less toxicity to both adult and children. The findings revealed that children are more likely to be more affected when cowpea controlled with agrochemical pesticide are consume (Table 9).

4. DISCUSSION

The soil physiochemical analysis shows that the use of synthetic pesticides is most likely the cause of low pH (4.25) value in the soil with agrochemicals compared with the *Aza* and *Bt* solution. Agro pesticide soil pH was somewhat acidic, falling below the specified range (6.5-8.5) for agriculture farming [40], whereas the soil with neem solution, *Bt* and control soil was within the acceptable range (Table 1). There was a significant difference between soil applied with agrochemical and the soil applied with alternative methods of pest control (*Aza and Bt*) ($p<0.05$). The physicochemical properties of soil are altered by chemical application, especially in soils where agrochemical pesticides are applied for cowpea pest control. This leads to an increase in heavy metals in the soil, which is likely passed on to plants that grow on such soils, providing long-term toxicity risks to humans and other ecosystem biota when consume. The agrochemical pesticides did the least damage to

cowpea leaves and bud, due to its effectiveness. There were significant variations between the pesticide and biopesticide use and control samples, due to their efficacy, which reduces pest effect on *Vigna.* The number of spots decreases over time, presumably due to the insecticide employed as well as the drying/hardening of the pods. Despite agrochemical effectiveness, it lead to the accumulation of harmful substances in the seed and soil, providing a health risk to humans (Table 2).

An earlier study showed that exposure to metal causes plants to produce reactive oxygen species [43, 44]. The current findings demonstrate that the amount of lipid peroxidation in the seeds of cowpea seedlings exposed to soil treated with agrochemical products increased as the quantity of agrochemical products in the soil rose (Fig. 2). Plants exposed to metal ions have been observed to have higher levels of lipid peroxidation [44-46]. High reactive oxygen species levels cause lipid peroxidation, which leads to oxidative stress [47]. It is important to note that agrochemical products may cause oxidative stress in exposed cowpea and decrease the level of antioxidants such superoxide dismutase, catalase, and xanthine oxidase activities as a result of increased generation of reactive oxygen species. Stress can increase the creation of reactive oxygen species, which can be dangerous to cells. However, plants have built-in defenses against reactive oxygen species. Superoxide dismutase (SOD) and catalase are two of the scavenging enzymes found in plants [48-50].

The antinutrients analysis showed that the cyanogenic glycoside, phytate, oxalate, alkaloid and tannin content in Cowpea samples that was controlled by agrochemical were higher than that of the biopesticides (*Aza* and *Bt*) and control sample which indicates that the cowpea controlled with agrochemical led to a significant ($p < 0.05$) increased of antinutrient factors in the cowpea seed. High concentration of cyanogenic glycoside stop cells from using oxygen and eventually causes heart, respiratory and central nervous problem [16]. Phytate, oxalate and tannins decreases the bioavailability of macromolecules (proteins, carbohydrate) and essential elements (calcium, magnesium, zinc, iron, and phosphorus). They form insoluble complexes, such as calcium oxalate crystals when binds to calcium and this complexes are not readily absorbed by the gastrointestinal tract which lead to health problems such as kidney stone oxalemia [51]. Alkaloids cause infertility, gastrointestinal and neurological disorder [15].

The metal levels discovered in the soil were all within the FAO/WHO soil permissible limit. According to analysis of variance (ANOVA) conducted, the concentrations of the hazardous elements in the soil varied significantly ($p <0.05$). In comparison to all metals analyze on soil, Pb content had the highest value in cowpea with agrochemical application. Regular consumption of Pb-contaminated foods has been shown to affect the liver, kidneys, heart, brain, nerves, and other vital organs. Pb exposure can cause heart disease, anemia, high blood pressure, and reproductive problems such osteoporosis (brittle bone disease), especially in men. Heavy metal concentrations in soil were found to be in the following order: Pb >Cr> As > Cu > Hg >Cd. The soil heavy metals were all below the WHO/FAO permissible limit, with the exception of mercury (4.48 mg/kg), which had an allowable limit of 2.0 mg/kg. Symptoms of mercury toxicity include, memory loss, headaches, hair loss, mental retardation in the fetus, fetal abnormalities, blindness, deafness, and muscle rigidity [52, 53]. Similarly, [14] discovery have shown that *Aza* and *Bt* has a lower heavy metal content in the soil than synthetic agrochemicals which is in line with this current research.

In the *Vigna u* seed and blood sample examined for heavy metals, the cowpea and blood with agro pesticide had higher heavy metals levels, and they are all above the WHO/FAO authorized limit of metal in blood and cowpea. The differences were significant ($p<0.05$) when compared to the cowpea controlled *Aza* and *Bt* solution. Heavy metals and nutrients received by the roots are often translocated to other parts of the cowpea, including the leaves and seed. On the other hand, metal availability in the soil and continual absorption by the roots could lead to higher concentrations in various areas of the cowpea. The amounts of heavy metals in *Vigna u* controlled for pest with an agricultural agropesticide decreased in the following order: Pb > Cr > As > Cu > Hg > Cd. The metal Pb showed the highest concentration (7.41 mg/kg) in the cowpea, exceeding the permissible limit (2.0 mg/kg) in agrochemical controlled cowpea. The rise in Pb and other metals could be linked to the widespread use of agricultural agropesticides. Little quantity of heavy metals are important for human health, large doses might cause metabolic disorders, according to [54]. According

to the CDC, Cd causes acute and chronic poisoning, as well as damage to the immune system [55]. High dosages of Cr have been related to chronic bronchitis, and vomiting, according to studies [56]. When the brain, nervous system, and red blood cells are exposed to high levels of Pb, it causes mental deterioration, decreased reaction time, memory loss, decreased fertility, renal system damage, nausea, insomnia, anorexia, and joint weakness [57].

Heavy metals were found in varying levels in the blood of rat fed with various pesticides, which might be attributable to the presence of these trace elements in pesticides sprayed on the cowpea. In prior analyses, metals were discovered in insecticides [58-60]. Following the heavy metals concentrations mean values in *Vigna u* that neem solution biopesticide were applied Cu > Pb > As > Cr > Hg > Cd. Cu and Hg, Cu (2.78) and Hg (0.27 mg/kg) exceeded the WHO/FAO permissible limits (0.04 and 0.1 mg/kg respectively). The presence of Cu and Hg in *Vigna u* samples with neem solution may be as a result of the atmospheric conditions/air deposition [61]. Other metals were found to be below the FAO/WHO permissible level in *Vigna u* with *Aza* and *Bt* biopesticide. In this study, the *Aza* and *Bt* biopesticide demonstrated non-significant level of metals to the soil or *Vigna u* seed. Neem solution and *Bt* has a low or no harmful effect on cowpea seed, and it is biodegradable when applied to plants. Metal absorption rate depend on soil physicochemical qualities, and other factors [62] could explain the discrepancies in metal accumulation in the cowpea plant under study (Table 5).

The BAF value of Cu and Pb (0.83) was found to be more in the *vigna u* controlled with agrochemical. Plants are known to take up and accumulate trace metals from contaminated soil via absorption from the root. The BAF of other elements are less than 1 and falls within the normal range of transfer. Hyper accumulators are plants having a BF greater than one, and they could be used in bioremediation of extremely polluted soil. The element is excluded from the soil by the *Vigna u* when the BAF is less than 1. The DIM values of heavy metals were greater in the *Vigna u* that had been sprayed with an agricultural agropesticide. When *Vigna u* is consumed, the DIM values obtained show the amount of metal that will be accumulated in a day for both adults and children. In this findings the HQ values for all heavy metals were significantly higher in *Vigna u* controlled with agrochemical pesticide than that of the *Aza* and *Bt*. The findings of HQ [27] show that Pb, As, and Cd pollution pose a significant health risk to both adults and children, but that Zn (1.058) exposure poses only a little harm to children who consume onions. The dangers of consuming polluted plants are more likely to affect children than adult. *Vigna u* controlled with agroagricultural pesticides is not safe for consumption, according to the findings of the hazard index.

5. CONCLUSION

This study has concludes that exposure of cowpea plant to agrochemical products in soil could impose oxidative stress and heavy metals in the blood when consume. The values of heavy metals and antinutrient constituents in *Vigna u* seed controlled with agrochemical pesticide were significantly higher and above WHO/FAO acceptable limits in cowpea. The risk assessment indicate that consumption of these *Vigna u* controlled with synthetic pesticides can pose a health risk as a result of heavy metal intake, especially to children. Neem and *Bt* biopesticide have shown as an alternative method in the management of cowpea pest.

AVAILABILITY OF DATA AND MATERIAL

We confirm the availability of all the data included in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sharma HC. Bionomics, host plant resistance, and management of the legume pod borer, *Maruca vitrata* (PDF). Crop Protection. 1998;7(5):373–386. DOI:10.1016/s0261-2194(98)00045-3.
2. Jayasinghe RC, Premachandra WTS. Dammini; Neilson Roy. A study on Maruca vitrata infestation of Yard-long beans (*Vigna unguiculata* subspecies sesquipedalis). Heliyon. 2015; 1(1):e00014. DOI:10.1016/j.heliyon.2015.e00014. PMC 4939760. PMID 27441212.
3. Yuguda AU, Abubakar ZA, Jibo AU, AbdulHameed A, Nayaya AJ. Assessment

of toxicity of some agricultural pesticides on earthworm (*Lumbricus terrestris*). American-Eurasian Journal of Sustanaible. Agriculture. 2015;9(4):49-59.

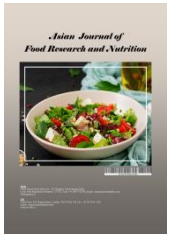
4. Achuba FI. Petroleum products in soil mediated oxidative stress in cowpea (*Vigna unguiculata*) and Maize (*Zea mays*) seedlings. Open Journal of Soil Science. 2014;4:417-435. DOI:http://dx.doi.org/10.4236/ojss.2014.412042.
5. Olowoyo JO, Okedeyi OO, Mkolo NM, Lion GN, Mdakane STR. Uptake and translocation of heavy metals by medicinal plants around a waste dumpsite in Pretoria, South Africa. South African Journal of Botany. 2011;78:116-121.
6. Mutune AN, Makobe MA, Abukutsa-Onyango MOO. Heavy metal content of selected African leafy vegetables planted in urban and peri-urban Nairobi, Kenya. African Journal of Environmental Science and Technology. 2012;8(1): 66-74.
7. Ravindran J, Pankajshan M, Puthur S. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdisciplinary Toxicology. 2016;9(3–4):90–100.
8. Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. Front. Plant Science. 2017; 8:610.
9. Kumar A, Singh M, Singh PP, Singh SK, Singh PK, Pandey KD. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in Curcuma longa L. Biocatal Agric Biotechnol. 2016;8:1–7. DOI:https://doi.org/10.1016/j.bcab.2016.07.002
10. Kumar P, Madhu K, Rituraj B, Dipendra KM, Bharti S. *Bacillus thuringiensis* as microbial biopesticide: uses and application for sustainable agriculture. Egyptian Journal of Biological Pest Control. 2021;31:95. DOI:https://doi.org/10.1186/s41938-021-00440-3
11. Kumar S, Vandana UK, Agrwal D, Hansa J. Analgesic, anti-inflammatory and anti-pyretic effects of *Azadirachta indica* (Neem) leaf extract in albino rats. International Journal of Science Research. 2015;4:713–721.
12. Kwasi OB, Samuel KT, Michael AA, Jerome DK. Production of natural insecticide from Neem leaves (*Azadirachta indica*). Asian Journal of Plant Science and Research. 2011;1(4):33-38.
13. Rhoda B, Freyer B, Macharia J. Towards reducing synthetic pesticide imports in favour of locally available botanicals in Kenya: Conference on International Agricultural Research for Development. 2006;11–13.
14. Oguh C. Egwu, Musa A. Dickson, Orum T. Gabriel, Iyaji R. Okai, Musa Amanabo. Risk assessment of heavy metals level in soil and jute leaves (*Corchorus olitorius*) Treated with *Azadirachtin* neem seed solution and organochlorine. International Journal of Environment, Agriculture and Biotechnology (IJEAB). 2019a;4(3):256-266. DOI:http://dx.doi.org/10.22161/ijeab/4.3.24.
15. Awomukwu DA, Nyananyo BL, Ikpeama AI, Adieze CU. Comparative chemical constituents of some cassia species and their pharmacological importance in South Eastern Nigeria. Science Journal of Chemistry. 2015;3(3):40-49.
16. Elllenhom MJ, Barcelonx DG. Medical toxicology; Diagnosis and treatment of human poisoning. Elsevier Science Publishing Co. New York, USA; 1988.
17. Agbaire PO, Oyewole A. Levels of anti-nutritional factors in some common leafy edible vegetables of southern Nigeria. Journal of Food Science Technology. 2012;3:99-101.
18. Olayemi FO. Evaluation of the reproductive and toxic effects of *Cnestis ferruginea* (de candolle) root extract in male rats. Journal of Pharmacology and Toxicology. 2007;33:46 – 51.
19. Dei HK, Rose SP, Mackenzie AM. Shea nut (*Vitellaria paradoxa*) meal as a feed ingredient for poultry. World's Poult Science Journal. 2007;63(4):611 – 624.
20. Nwogu LA, Igwe CU, Emejulu AA. Effects of *Landolphia owariensis* leaf extract on the liver function profile and haemoglobin concentration of albino rats. African Journal Biotechnology. 2008;2(12): 240-242.
21. Osuji CA, Ugwu OC, Oguh CE, Augustine O, Ejiofor UM. Parasitological Analyses and Soil physicochemical properties in African Giant Land Snail (*Archachatina margenata*) reared with dump soil. Advanced Journal of Environmental Science and Technology (AJEST). 2021;. 6(1):259-265.

22. Misra HP, Fridovich I. The role of superoxide in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. Biochemical Journal. 1972;247:3170-3175.
23. Aksnes A, Njaa RL. Catalase, glutathione peroxidase and superoxide dismutase in different fish species. Comparative Biochemistry and Physiology. 1981;69B:893-896.
24. Rani P, Meena Unni, K, Karthikeyan J. Evaluation of Antioxidant Properties of Berries. Indian Journal of Clinical Biochemistry. 2004;19:103-110.
25. Association of Official Analytical Chemist (AOAC). Official methods of Analysis, 15th edition, Washington; 1995.
26. Pearson D. The chemical analysis of foods. 7th edition, Churchill, livingstone. 1976;493.
27. Barau BW, Abdulhameed A, Ezra AG, Muhammad M, Kyari EM. Heavy metal contamination of some vegetables from pesticides and the potential health risk in Bauchi, Northern Nigeria. International Journal of Science and Technology. 2018;7 (1):1-11.
28. Olowoyo JO, Van Heerden E, Fischer JL, Baker C. Trace metals in soil and leaves of *Jacaranda mimosifolia* in Tshwane area, South Africa. Atmospheric Environment. 2010;44(20): 1826–1830.
29. Olowoyo JO, Lion GN. Population health risk due to dietary intake of toxic heavy metals from *Spinacia oleracea* harvested from soils collected in and around Tshwane, South Africa. South African Journal of Botany. 2013;88(11):178–182.
30. Egwu OC, Jennifer UO, Goretti ACM, Uchechukwu O, Marksydney EU. toxic elements and microbial loads in african giant land snail (*Archachatina margenata*) reared with waste contaminated soil. Applied Research in Scince and Technology. 2021;1(1): 26-35.
31. USEPA. Multimedia, Multi-pathway and Multi-receptor Risk Assessment (3MRA) Modellling System. U.S Environmental Protection Agency, Office of Research and Development, Washington DC. 2002;1-9.
32. Fowomola MA. Some nutrients and antinutrients components of mango (*Mangifera indica*) seed. African Journal of Food Science. 2010;4(8):472 – 476.
33. Abdoulaye C, Brou K, Jie C. Phytic acid in cereal grains: structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. American Journal of Plant Nutrition and Fertilization Technology. 2011;1(1):1-22.
34. Pearson D. The chemical analysis of foods. 7th edition, Churchill, Livingstone. 1976;493.
35. Adhikari KM, Sweetingham MW, Buirchell B. Yellow lupin breeding in Western Australia. Proceedings of Agribusiness Crop Updates, Lupins and Pulses. 2005;12–14.
36. Laconelli S, Simmen B. Cite as taste thresholds and suprathreshold responses to tannin-rich plant extracts and quinine in a primate species (*Microcebus murinus*). Journal of Chemical Ecology. 2002;28(11):2315–2326.
37. WHO/FAO. *Codex alimentarius* commission. Food additives and contaminants. Joint FAO/WHO Food Standards Programme, ALINORM 10/12A; 2001.
Available:www.transpaktrading.com/static/pdf/research/achemistry/introTofertilizers.pdf
38. Oguh CE, Uzoefuna CC, Ugwu CV, Ubani CS, Musa AD, Okunowo WO. Evaluation and ecological risk assessment of selected heavy metal pollution of soils and *Amaranthus cruentus* and *Telfairia occidentalis* grown around dump site in Chanchaga Minna, Niger State, Nigeria. Asian Journal of Environment & Ecology. 2019b;10(2):1-16.
DOI:http://dx.doi.org/10.9734/ajee/2019/v10i230114.
39. WHO/FAO. Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission 10th Session. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF (Prepared by Japan and the Netherlands) 4 - 8 April 2016; 2016.
40. FAO/WHO. Toxicological evaluation of certain food additives and food contaminants. (Twenty-eight meeting of the Joint FAO/WHO Expert Committee on food additives). Washington, DC: ILSI Press International Life Sciences Institute; 1984.
41. Goullé JP, Le Roux P, Castanet M, Mahieu L, Guyet-Job S, Guerbet M. Metallic profile of whole blood and plasma in a series of 99 healthy children. J Anal Toxicol. 2015;39:707–13.

42. Center for disease control. Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables. January 2017;1.
Available:https://www.cdc.gov/exposurereport/index.html
Accessed 11 Jan 2018.
43. Hartley-Whitaker J, Ainsworth G, Mehary AA. Copper and arsenate-induced oxidative stress in *Hocuslanatus* L. clones with differential sensitivity. Plant Cell and Environment. 24:713-722.
DOI:http://dx.doi.org/10.1046/j.0016-8025.2001.00721.x
44. Somashekaraiah BV, Padmaja K, Prasad ARK. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. Physiologia Plantarum. 1992;85:85-89.
DOI:http://dx.doi.org/10.1111/j.1399-3054.1992.tb05267.x
45. Gallego SM, Benavides MP, Tomaro ML. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Science. 1996;121:151-159.
DOI:http://dx.doi.org/10.1016/S0168-9452(96)04528-1
46. Lozano-Rodriguez E. Hernandez CE, Bonay P, Carpena-Ruiz RO. Distribution of cadmium in shoots and root tissues of maize and pea plants: physiological disturbances. Journal of Experimental Botany. 1997;48:123-128.
DOI:http://dx.doi.org/10.1093/jxb/48.1.123
47. Frei B. Reactive oxygen species and antioxidant vitamins: mechanism of action. American Journal of Medicine. 1994;97:S5-S13.
DOI:http://dx.doi.org/10.1016/0002-9343(94)90292-5
48. Asada K, Takahashi M. Production and scavenging of active oxygen in chloroplasts. In: Kyle, D.J, Osmond, C.B. and Arntzen, C.J, Eds, Photoinhibition, Elsevier, Amsterdam. 1987;227-287.
49. Bowler C, Van Montague M, Inze D. Superoxide dismutase in plants. Critical Review of Plant Science. 1994;13:199-218.
DOI:http://dx.doi.org/10.1080/07352689409701914
50. Jayakumar K, Jaleel AC, Viayarengan P. Changes in growth, biochemical constituents and antioxidant potentials in radish (*Raphasus sativus* L.) under cobalt stress. Turkish Journal of Biology. 2007;31:127-136.
51. Akande FO, Ajayi SA. Assessment of heavy metals level in soil and vegetables grown in peri-urban farms around Osun State and the associated human health risk. International Journal of Environmental, Agriculture and Biotechnology. (IJEAB). 2017;2(6):2456-1878.
DOI:http://dx.doi.org/10.22161/ijeab/2.6.61.
52. Clarkson TW, Magos L, Myers GJJ. The toxicology of mercury: Current exposures and clinical manifestations. New England Journal of Medicine. 2003;349:1731-1737.
53. Oguh CE, Uzoefuna CC, Ugwu CV, Ubani CS, Musa AD, Okunowo WO. Evaluation and ecological risk assessment of selected heavy metal pollution of soils and *Amaranthus cruentus* and *Telfairia occidentalis* Grown around Dump Site in Chanchaga Minna, Niger State, Nigeria. Asian Journal of Environment & Ecology. 2019b;10(2):1-16.
DOI:http://dx.doi.org/10.9734/ajee/2019/v10i230114.
54. Dixit R, Malaviya D, Pandiyan K, Singh UB, Sahu A, et al. Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. Journal of Sustainability. 2015;7:2189–2212.
55. Jabeen S, Shah MT, Khan S, Hayat MQ. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. J. of Med. Plants Res. 2010;4(7): 559–566.
56. Barakat M. New trends in removing heavy metals from industrial wastewater. Arab Journal of Chemistry. 2011;4(1):361–377.
57. Nagajyoti P, Lee K, Sreekanth T. Heavy metals, occurrence and toxicity for plants: A review. Environmental Chemistry. 2010;8(1):199–216.
58. Nazir R, Khan M, Masab M, Rehman HU, Rauf NU, et al. Accumulation of heavy metals in the soil, water, and plants, and analysis of physico-chemical parameters of soil and water collected from Tanda Dam, Kohat. Journal of Pharmaceultical Science and Research. 2015;7(3): 89-97.
59. Fonge BA, Nkoleka EN, Asong FZ, Ajonina SA, Che VB. Heavy metal contamination in soils from a municipal landfill, surrounded by banana plantation in the eastern flank of Mount Cameroon African. Journal of Biotechnology. 2017;16(25):1391-1399.

60. Nimyel DN, Egila JN, Lohdip YN. Heavy metal concentrations in some vegetables grown in a farm treated with urban solid waste in Kuru Jantar, Nigeria. British J. of Applied Sci. and Technol. 2015;8(2):139-147.
61. Luo C, Liu C, Wang Y, Liu X, Li F et al. Heavy metal contamination in soils and vegetables nearan e-waste processing site, south China. Journal of Hazardous Materials. 2011;186(1): 481–490. DOI:10.1016/j. jhazmat.2010.11.024
62. Alloway BJ. The origin of heavy metals in soils. In Alloway, B. J. (Ed). Heavy metals in soils. Blackie, Glasgow and London. 1990;29-39.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97723



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 74-88, 2023; Article no.AJFRN.98418

Assessing Eating Habits, Physical Activity, and Nutritional Knowledge among Female Adolescents in Saudi Arabia

Azzah Alsheweir [a*]

[a] *Applied Medical Sciences College, King Saud University, Saudi Arabia.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/98418

Original Research Article

Received: 18/02/2023
Accepted: 20/04/2023
Published: 02/05/2023

ABSTRACT

Studies on the health and nutrition of the Saudi population, especially those of children and adolescents, are limited. Assessing Saudi adolescents in relation to essential nutrition concepts is needed. This study examined nutrition related variables and evaluated factors influencing the eating habits of Saudi adolescent girls. It compared nutrition and lifestyle between two distinct locations in Saudi Arabia. A validated dietary questionnaire was given to 291 adolescent females students (aged 14-18 years) attending intermediate and high schools. The mean age for the sample was 14.6 ± 3.5 years. The majority of females were considered within normal weight (60%). Eating habits were significantly healthier among Al-Khobar females compared to Riyadh females (P = 0.028). Similarly, females from Al-Khobar reported being more physically active (P = 0.002) with higher self-efficacy (P = 0.049) compared with females from Riyadh. No significant differences were found among BMI categories. Bivariate correlation analyses found that eating habits were correlated with self-efficacy (P < 0.001) and knowledge of healthy dietary habits (P = 0.001) but were not associated with nutrition knowledge (P = 0.09). A linear regression model demonstrated

**Corresponding author: E-mail: mawhobon1@gmail.com;*

that predictors—location (P = 0.028), self-efficacy (P < 0.001), and knowledge of healthy dietary habits (P = 0.008)—explained 12.8% of the variance. Location, self-efficacy, and knowledge of healthy dietary habits were significant predictors relating to eating habits. Educational strategies to improve nutrition and physical activity are needed to help female adolescents achieve lifelong healthy eating and active lifestyle behaviors.

Keywords: Eating habits; physical activity; adolescents; nutrition knowledge; self-efficacy; Saudi Arabia.

1. INTRODUCTION

Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year because of being overweight or obese [1]. Worldwide, at least 10% of school-aged students are considered overweight or obese with the majority in the Americas (32%) followed by Europe (20%) and the Middle East (16%) [2].

In Saudi Arabia, evidence from serial cross-sectional assessments of body mass index (BMI), or percent body fat, on Saudi children and adolescents have confirmed a rising trend in obesity over the last two decades [3]. It has been estimated that among adolescents aged 13–18 years 26.6% are overweight and 10.6% are obese [4]. Moreover, adolescent girls have higher combined overweight and obesity prevalence rates compared with adolescent boys in Saudi Arabia [5].

Adolescence is a transitional period where young people are determining and taking control of their own eating habits and health behaviors [6]. Unfortunately, there is an increased trend toward unhealthy eating habits among adolescents, such as skipping breakfast and consuming a great deal of soft beverages [7,8]. A recent study investigating the relationship of adolescents' behaviors to physical and psychological health found that the majority of students (47.2%) reported low physical activity, low fruit and vegetable intake, and a high intake of sweets, chips, and fries. Approximately 26.5% reported high physical activity, high fruit and vegetable intake, and a low intake of sweets and soft drinks [9].

Similar trends of consuming more animal products and refined foods at the expense of vegetables and fruits have been reported for adolescents in Saudi Arabia [10,11]. The majority of Saudi adolescents (ranging from 66.8% to 80.4%) did not consume the daily Dietary Guidelines Recommendations (DGR) for milk, fruit, and vegetables. Adolescents did not consume breakfast on a regular basis. Unhealthy food types such as French fries, potato chips, cakes, donuts, candy, and chocolate were most often consumed with a higher prevalence of candy and chocolate consumption among females (52% for females as opposed to 37% for males) [12].

Even though the reasons for the recent and dramatic nationwide increases in overweight and obesity in children and adolescents are unclear, this trend can be attributed to the production and frequent consumption of convenience foods that are high in fat and calories (e.g., candy, chips, and sugary drinks) and diminished physical activity compared to the past [13-15].

Within the past decades, the Kingdom of Saudi Arabia, as well as other Arabian Gulf countries, has undergone tremendous lifestyle changes that include physical activity patterns and eating habits. Such dramatic lifestyle changes are thought to have contributed immensely to the increase in obesity prevalence among Saudi children and youth [16]. The intake of animal products and refined sugar has increased while the intake of fruit and vegetables and complex carbohydrates has decreased [17]. Furthermore, sedentary lifestyles are becoming particularly commonplace among Saudi children and youth [18].

Certain dietary patterns are noticeably related to adolescent obesity [19]. Skipping breakfast is a major dietary habit in Saudi Arabia and is positively correlated with obesity [20]. Approximately 74% of Saudi schoolgirls aged 12-16 years skipped or irregularly consumed breakfast [21]. According to Al-Hazza et al. [12], the number of Saudi adolescents skipping breakfast ranged from 15% to 49%.

Less healthy snacking can be another factor linked to obesity. Many studies reported that carbonated beverages, cheese, non-carbonated canned drinks, candy, chocolate, and potato chips were foods commonly consumed as snacks among adolescents [12,21].

Ordering meals, eating out, or consuming ready-to-eat foods are behaviors that have become prevalent in Saudi Arabia. Musaiger [21] reported that high intake of fast foods and decreased consumption of homemade meals are associated with obesity among children and adolescents in Saudi Arabia and other Gulf countries. Moreover, he concluded that the incidence of obesity is expected to reach 52% or more among Saudi children who eat outside the home five times or more per week.

Physical inactivity and sedentary lifestyles are becoming more prevalent among Saudi children and adolescents. A study conducted in Al-Khobar, Jeddah, and Riyadh concluded that about 50% of males and 75% of the females did not meet daily physical activity guidelines. Females were more sedentary and less physically active. Almost 84% of males and 91.2% of female Saudi adolescents spent more than two hours daily onscreen (e.g., watching television) [12]. Another study revealed that normal weight adolescents were more active than obese ones, and participants with higher BMI reported lower levels of physical activity and higher amounts of sedentary time [22]. In addition, higher levels of physical activity were found among males when compared to females; however, physical activity levels appeared to decline with age for both groups.

Self-efficacy is another factor that has to be assessed as a critical determinant of behavior change in relation to health promotion and disease prevention. According to Bandura [23], self-efficacy is the internal belief about the ability to organize and execute courses of action necessary to achieve a goal. Therefore, individuals with strong self-efficacy beliefs are more confident in their capacity to execute a behavior. Most of the widely known health behavior theories include self-efficacy or similar concepts [24-26]. Preventive nutrition, dieting, weight control, and physical exercise can be guided by nutrition and physical exercise self-efficacy beliefs [27]. A study examined adolescent girls aged 18-21 years in Jeddah (western region of Saudi Arabia) in relation to knowledge, attitude, and behavior regarding fruit and vegetable consumption using a Transtheoretical Model (TTM). It was found that self-efficacy and pros were the most significant positive predictors of adopting healthy dietary habits such as fruit and vegetable consumption [28].

Nutrition knowledge is one of the factors influencing dietary practices. Inappropriate nutrition education and poor nutrition knowledge increase the possibility of developing poor dietary practices [29]. A study conducted to determine the correlation between nutrition knowledge and dietary behaviors found that knowledge was significantly associated with healthy eating: respondents with higher levels of nutrition knowledge are about 25 times more likely to meet current recommendations for fruit, vegetable, and fat intake than those with lower levels of knowledge [30]. According to Al-Almaie [31], knowledge about healthy diets was not adequate among Saudi school adolescents. This cross-sectional study included 1,240 males and 1,331 females from secondary schools in Al-Khobar, a mid-size city located in the eastern province of Saudi Arabia. About 51% of the male and 65% of the female students recognized unsaturated fats as healthy foods. However, students' knowledge on the benefits of fiber-containing foods and the risks of unhealthy foods was disappointing. Preliminary observations to assess nutrition awareness among 311 Saudi adolescent and adult subjects in Riyadh, the largest city in Saudi Arabia and located in the central region, concluded that an acceptable number of them consumed a variety of foods from the four food groups every day, which reflected a good trend in food and nutrition awareness [32].

The Saudi Arabian population has experienced major transitions in eating patterns and life-styles due to economic changes and rapid urbanization. Findings among adolescents show higher percentages of negative eating practices and reduced physical movement. In relation to nutrition knowledge, limited references concluded that the levels of knowledge among adolescents in Saudi Arabia were not adequate to promote positive eating patterns. Self-efficacy is confirmed to be associated positively with healthy diet patterns and physical activity.

The objectives of this study were to [1] examine eating habits, physical activity, self-efficacy, awareness of healthy dietary habits, and nutrition knowledge among adolescent girls aged 14-18 years, [2] compare these variables between Riyadh and Al-Khobar, and [3] determine the factors affecting dietary habits.

In general, studies of the Saudi population including children and adolescents are limited. For this reason, assessing Saudi adolescents in

relation to some essential health concepts appears to be needed. Locally, this study determined the quality of dietary consumption associated with the levels of nutritional knowledge among Saudi female adolescents. This information can be used as a reference to plan nutrition awareness programs, to improve nutrition consciousness and to promote the adoption of healthy lifestyles. Internationally, this study can aid in generating an introductory background about Arab and Middle Eastern populations. Further comprehensive studies that compare Saudi Arabia with other countries can be established.

2. METHODS

A cross-sectional survey was developed and administered to female intermediate and high school students randomly selected from two specific locations in Saudi Arabia (Riyadh and Al-Khobar) to assess their eating habits, physical activity, self-efficacy, and nutritional knowledge. The research protocol was approved by the Institutional Review Board at California State University Chico (CSUC) in May 2013 prior to implementation.

2.1 Participants

Participants for this study were 291 female students aged 14-18 years from intermediate and high schools in Riyadh and Al-Khobar. After a random selection of schools, the principals were contacted for approval to recruit students and distribute self-administered dietary questionnaires. Teachers received instructions regarding student consent. Under teacher supervision, surveys were distributed to students who were given 10-15 minutes to complete. Completed surveys were collected by teachers and given to the first author.

2.2 Study Locations

For this study, data were collected in two locations of Saudi Arabia: Riyadh and Al-Khobar. These locations were selected so more than one population area could be studied and to develop a more diverse representation of the Saudi population.

Riyadh: Riyadh is the largest city in Saudi Arabia with a population of 5.3 million people [33]. It is located in the center of the Arabian Peninsula with an area of 400 square miles. Riyadh is one of the richest urban cities in the Middle East and is the heart of economic and industrial development in Saudi Arabia. It is experiencing numerous and massive improvements in different areas of its society including educational, financial, agricultural, technical, and social programs. Eating patterns have changed dramatically with the population preferring processed and fast foods to home cooked meals, especially among children and adolescents. Moreover, Riyadh has a hot desert climate during summer with little rainfall. This hot, dry weather is the main impediment to Saudis engaging in outdoor activities (e.g., walking and jogging).

Al-Khobar: Al-Khobar is a mid-size city located in the eastern province of Saudi Arabia on the coast of the Arabian Gulf, about 250 miles northeast from Riyadh. The population is estimated to be 250,000 consisting of both Saudi citizens and international expatriates [34]. It is a vibrant business center that houses the world's largest oil company (Saudi Aramco). The weather tends to be very hot and humid during summer but dry and cool in winter. As an important region of Saudi Arabia, Al-Khobar has also experienced noticeable improvements within the past decades in several areas. Unlike Riyadh's residents, the citizens of Al-Khobar engage in many water sports and activities since they are on the east coast.

2.3 Surveys

A self-administered dietary questionnaire, originally developed for adolescent students in Italy [35], was used for this cross-sectional study. This questionnaire was previously constructed and tested for reliability [35,36]. Considering the major concepts identified in this cross-sectional study, the dietary questionnaire used by the Italian researchers had to be modified for this study.

The distributed questionnaire was translated into Arabic by the first author. Some questions were modified or removed to meet the religious and cultural requirements of the Saudi population. In particular, questions containing food items such as pork or alcoholic drinks were changed to other allowed food items. Also, specific Italian dishes (e.g., tiramisu) were replaced with food items better known to the Saudi population. There were six sections in the revised questionnaire.

Section 1. Personal Information: This section contained information on personal data: age, weight in kilograms, and height in centimeters.

Self-reported weight and height were used to calculate BMI. The English version of the children's BMI metric calculator was used [37]. Date of measurement, weight, and height were entered for each participant to obtain BMI and BMI-for-age percentile (%ile) values.

Section 2. Eating Habits: This section had 13 questions investigating the dietary habits of the adolescents. Questions asked about the number of meals per day, breakfast content, daily consumption of fruit and vegetables, and typical consumption of water and other beverages. Seven questions had the following response categories: always, often, sometimes, never. The other six had four structured response categories that correspond to each question. The scores ranged from 0 to 3 with the highest score assigned to the healthiest behavior and the lowest score to the least healthy behavior. The total score for this section was 39: low scores indicated "inadequate eating habits" and high scores indicated "satisfactory eating habits."

Section 3. Physical Activity. This section had five questions assessing the levels of physical activity. The responses were structured differently according to each question. The scores ranged from 0 to 3 with the maximum score assigned to the healthiest behavior and the minimum score to the least healthy behavior. The total score for this section was 15: low scores indicated "sedentary physical level" and high scores indicated "active physical level."

Section 4. Self-efficacy: This section had eight questions investigating the personal behaviors and attitudes linked to improving health status in relation to nutrition. These questions had three response categories: no = 0, I don't know = 1, yes = 2. The total score for this section was 16: low scores indicated "incapacity for using advice aimed at improving one's wellbeing" and high scores indicated "good capacity for using advice aimed at improving one's wellbeing."

Section 5. Awareness of Healthy Dietary Habits: This section had eight questions investigating the levels of knowledge in relation to modifications or improvements in eating habits. Each question had two responses: Yes=0, No=1. The total score for this section was 8: low scores indicated "high levels of knowledge of modifying one's own eating habits with the aim of improving them" and high scores indicated "low levels of knowledge of modifying one's own eating habits with the aim of improving them."

Section 6. Nutritional Knowledge: This section had 11 questions investigating students' knowledge regarding particular food items and various nutritional definitions. Each question had four different response categories. The scoring was 1 for correct answers and 0 for wrong answers. The total score for this section was 11: low scores indicated "insufficient nutritional knowledge" and high scores indicated "good nutritional knowledge."

2.4 Statistical Analysis

Responses from these questionnaires were expressed as means, standard deviations, and distribution of scores. Independent t-tests were computed to investigate the differences in scores among study locations. One-way ANOVA was computed to compare score differences among three BMI groups (underweight, normal, and overweight/obese). Bivariate analyses were tested to determine the nature of the association between eating habits and other variables. Linear regression was performed to identify the significant factors affecting dietary habits. Microsoft Excel was used to code the variables. SPSS software version 19.0 (SPSS, Inc., Chicago, IL) was used to perform all statistical analyses. Participant recruitment and questionnaire distribution were held throughout May 2013. Data entry and analyses were conducted throughout the fall of 2013. $P \leq 0.05$ was considered statistically significant and all P values were two sided.

3. RESULTS

3.1 Characteristics

Anthropometric measurements for the sample are shown in Table 1. A total of 291 female students were recruited for this study: 160 participants (55%) participated from Riyadh and 131 (45%) from Al-Khobar. The majority of recruited female students were aged 14 years (34%) and 25% were aged 15 years. The mean age for the sample was 14.6 ± 3.5 years.

According to BMI-for-age percentiles, most females were considered within normal weight (60%) while 28% were found to be underweight. Only 10 participants were classified as obese and 24 were overweight. The mean weight for the study population was 52.2 ± 10.7 kg.

3.2 Eating Habits and Physical Activity

The mean score obtained for eating habits was 21.7 ± 5. Skipping breakfast was not prevalent in

the sample (6.5%). Additionally, 59.4% of the female adolescents indicated they did not consume milk or yogurt at breakfast, and 23.7% and 21% of the sample did not consume at least two portions of fruits and vegetables per day, respectively. About 27% of the sample indicated a high consumption of desserts and cakes, particularly during a meal (Table 2).

The mean score calculated for physical activity was 6.2 ± 2.9. In response to the question "do you usually practice a physical activity," 21.8% of the sample answered "always." Almost 14% of the females responded "never." In response to the question "what do you prefer to do during your free time," 73.6% of the sample answered "watching television," "using the computer," "listening to music," or "reading a book." Only 8.7% and 8% answered "walking" and "practicing a sport," respectively (Table 2). Mean scores and standard deviations for self-efficacy, awareness of healthy dietary habits, and nutrition knowledge are shown in Table 3.

3.3 Nutrition Related Variables and Location

Table 3 shows the mean values of female adolescents' scores in relation to location (Riyadh and Al-Khobar). Eating habits were found to be significantly healthier in Al-Khobar compared to Riyadh ($t(253) = -2.21$, $P = 0.03$). Females from Al-Khobar also reported being more physically active compared with females from Riyadh ($t(281) = -3.20$, $P = 0.002$). Self-efficacy was also higher among the Al-Khobar population ($t(266) = -0.43$, $P = 0.049$). No differences were found for knowledge of healthy dietary habits and nutrition knowledge. Additionally, no significant differences in BMI categories were found between these two locations.

3.4 Nutrition Related Variables and BMI Groups

Table 4 shows the mean values of female adolescents' scores in relation to BMI groups (underweight, normal weight, overweight/obese). Across all survey sections, no significant differences were found among BMI groups.

3.5 Factors Affecting Eating Habits

Bivariate correlation analyses (Pearson's) were tested among eating habits responses and certain factors. Nutrition knowledge was not associated with eating habits ($r(224) = 0.113$, $P = 0.09$), but awareness of healthy dietary habits was negatively correlated with eating habits ($r(236) = -0.221$, $P = 0.001$). Using Spearman's test, self-efficacy was significantly related to eating habits ($r(239) = 0.270$, $P < 0.001$).

Three variables were tested for correlations with eating habits: location, self-efficacy, and knowledge of healthy dietary habits (Table 5). These variables were added in one linear regression model. The results indicated that the three predictors explained 12.8% of the variance ($R = 0.36$, $F(3,226) = 11.08$, $P < 0.001$). Location ($\beta = 1.39$, $P = 0.028$), self-efficacy ($\beta = 0.45$, $P < 0.001$), and awareness of healthy dietary habits ($\beta = -0.53$, $P = 0.008$) were significant predictors of eating habits.

4. DISCUSSION

This study assessed eating habits, physical activity, self-efficacy, awareness of healthy dietary habits, and nutritional knowledge among Saudi female students aged 14-18 years from two major geographical locations in Saudi Arabia. Most of the female adolescents had normal body weight or were underweight; only 10% of the females were classified as overweight or obese.

In this study, skipping breakfast was not a prevalent dietary pattern in the sample. Almost 93% of female adolescents reported consuming breakfast daily or frequently. These findings are not consistent with conclusions from other studies on the Saudi population [12,21]. Abalkhail et al. [38] found that skipping breakfast was reported by 14.9% of students and this habit did not differ by age, sex, body mass index, or social class. Among students aged 16-25 years in Riyadh, breakfast was skipped by 20% of respondents [39]. Another study found that breakfast was a regular meal for 49% of secondary school students and milk was consumed daily by 51.5% of the sample [40].

In our study, a small proportion of females consumed fruit and vegetables daily. Economic improvements in Saudi Arabia have encouraged negative eating behaviors. The majority of children and youth from Arab Gulf countries consume insufficient amounts of fruits and vegetables, which in turn can lead to inadequate dietary fiber and essential nutrient intake [41]. Many previous studies reported low consumption

of fruits and vegetables by both genders. Al-Rethaiaa and colleagues [10] reported that Saudi students do not frequently consume vegetables and fruits, except for dates. Unhealthy dietary habits are common in youth. Results from the 2009 National Youth Risk Behavior Surveillance study indicated that during the seven days preceding the survey, 78% of high school students had not eaten fruits and/or vegetables five or more times per day [42].

Physical inactivity was prevalent among females of this study. About 17% of females reported engaging in a particular sport. Physical inactivity associated with unhealthy diets are among the leading causes of major non-communicable diseases, including cardiovascular disease, type-2 diabetes, and certain types of cancer, which contributes substantially to the global burden of disease, death, and disability in Arab countries [43]. Saudi Arabia has witnessed significant lifestyle changes in recent years. Rapid urbanization, predominance of the automobile for personal travel, introduction of labor-saving devices in the home and the workplace, availability of high-fat and caloric-dense foods, satellite TV, and increased reliance on computers and telecommunication technology contribute to negative eating habits and poor physical activity patterns [12].

Screen time was the most reported sedentary behavior linked to physical inactivity and unhealthy eating: watching television takes away time that could be spent engaging in physical activities. This sedentary behavior increases food intake, especially unhealthy snacks, and decreases the motivation to move and be active. The prevalence of physical inactivity was significantly ($P < 0.001$) higher among Saudi adolescents (64%) compared to British adolescents (25.5%). The proportion of adolescents exceeding two hours of daily screen time was high among Saudis (88.0%) and British (90.8%) [44]. Similar findings revealed that 91.2% of female Saudi adolescents spent more than two hours onscreen daily [12]. A longitudinal study indicated opposing conclusions: it was shown that changes in watching television did not necessarily indicate changes in leisure time as the two behaviors represent two separate constructs, not functional opposites [45].

Self-efficacy is an essential concept influencing nutrition practices and dietary patterns [46]. This study confirmed the correlation between self-efficacy and dietary behavior. Using self-efficacy as a functional predictor for health behavior change is recommended. By implementing self-efficacy as a mediator variable in designed interventions, it will positively improve eating patterns and physical activity [47,48]. Planning an integrated dietary approach that uses the determinants of the TTM model has been proven effective among adolescents [28]. In addition, applying Bandura's Social Cognitive Theory constructs to a particular nutrition intervention has the potential to improve self-efficacy and overall health in adolescent females [49].

Study results revealed that adolescents' nutrition knowledge did not predict eating behavior. The literature on eating habits and nutrition knowledge were contradictory. Some have shown a positive and strong correlation between nutrition knowledge and eating behavior [31,32,46]. Other studies concluded only a small correlation exists between these factors [50]. Knowledge of what to eat is important in making healthy food choices but only if the knowledge is put into practice [51]. However, awareness of healthy dietary habits was associated with eating behavior in this study.

Significant locational variations in eating habits, physical activity, and self-efficacy were found. Al-Khobar adolescent females had significantly healthier dietary patterns and more active lifestyle compared to Riyadh adolescent females. Even though both cities have experienced rapid urbanization, Riyadh cannot be compared to Al-Khobar in terms of size and population density. Riyadh is considered the center of the industrial and business movement in Saudi Arabia, thus it is undergoing a stronger transition in eating patterns and lifestyle. Furthermore, extreme climate conditions limit outdoor activities during summer and winter in both cities [33,34]. However, Al-Khobar's geographical location on the east coast can serve as a major incentive to engage in certain physical activities compared to Riyadh's desert climate. Because of cultural and social reasons, Saudi females have fewer opportunities to engage in leisure-time physical activity, both inside and outside of school, compared with males [44].

Overweight and obesity were not common in our sample. These data are lower than reported by Mahfouz et al. [52], which showed that the rate of obesity and overweight amounted to 23.2% among boys and 29.4% among girls. Another cross-sectional study found that 61% of adolescent girls were normal weight, 28% were overweight or obese, and 11% were

underweight. Similar to our study, the findings show that adolescent girls in Saudi Arabia face two contrasting nutrition situations [53].

The rates of obesity are increasing among Saudi children and adolescents due to marked nutritional changes and rapid urbanization [5]. However, these trends tend to be the lowest among girls aged 14-16 years, which was seen in our study [4]. Al-Dossary et al. revealed that more than 50% of adolescents between 14 and 18 years had weight above the 85th percentile. However, only 19.2% of the female adolescents were considered overweight or obese [54].

Surprisingly, overweight and obesity were not related to scores obtained in the dietary questionnaire, even for eating habits and physical activity. In an international comparative study involving youth from 34 countries, a significant negative relationship was found between BMI categories and candy consumption in 91% of countries, while no association between the consumption of non-diet soft drinks and being overweight was found [55]. Recent studies about Saudi eating patterns and obesity revealed significant associations between unhealthy eating patterns and obesity [10,11,56]. Obese females were significantly less active (especially in terms of vigorous activity), had less favorable dietary habits (e.g., lower intake of breakfast, fruits, and milk), but had lower intake of sugar-sweetened drinks and sweets/ chocolates [56]. Al-Rethaiaa et al. [10] found a rising trend towards consuming more animal products and refined foods in the diet at the expense of vegetables and fruits. These eating patterns are correlated with an increased tendency of obesity and elevated body fat in children, adolescents, and adults in the past few decades.

In our model, eating habits were predicted by location, self-efficacy, and awareness of healthy dietary habits. Conceptual models and theories explain the dynamics of eating behavior and the surrounding external influences. Formulating a common theme based on these theories will help clarify the function of eating behavior according to personal and socio-environmental factors that interact to influence behavioral patterns [57]. In particular, Social Cognitive Theory (SCT) provides a useful framework for understanding the influential interactions related to eating behaviors in adolescents [57]. This theory examines a certain behavior in terms of interactions between personal factors, environmental aspects, and behavior.

Self-efficacy and knowledge are certain individual characteristics related to eating behavior. These intrapersonal factors have the ability to effectively modify the eating behavior [57]. Self-efficacy has frequently been a good predictor of health behavior, sometimes explaining 50% or more of variability [58]. Self-efficacy has found to be an associated variable in relation to predicting eating behavior among adolescents. Among adolescent girls, self-efficacy was identified as an important predictor for energy consumption, as it was inversely correlated with total intake at a meal ($P < .01$) [59]. Knowledge of healthy eating is essential, but knowledge by itself does not act towards adopting healthy eating behaviors in adolescents. A study based on twenty-three focus groups found that regardless of adolescents' knowledge, it is difficult to follow healthy eating recommendations and to limit consuming foods that perceived to be unhealthy [60-62].

Table 1. Anthropometric characteristics of the survey participants (n=291)

			Mean ± SD
Age	14	99 (34) [1]	
	15	73 (25) [1]	
	16	38 (13) [1]	14.6 ± 3.5
	17	43 (15) [1]	
	18	24 (8) [1]	
Weight			52.2 ± 10.7
Height			158.4 ± 6.6
BMI %ile[2]	Underweight	82 (28) [1]	
	Normal	175 (60) [1]	39.8 ± 33.5
	Overweight	24 (8) [1]	
	Obese	10 (3) [1]	

[1] *Values expressed as n (%)*

[2] *Body Mass Index (BMI)-for-age percentile, for children / adolescents 2 years and older*

Table 2. Distribution of female adolescents' responses for eating habits and physical activity

Eating Habits				
Q1: Do you eat breakfast? (n = 291)	Always 81 (27.8) [1]	Often 79 (27.1)	Sometimes 112 (38.5)	Never 19 (6.5)
Q2: Which beverage do you consume at breakfast? (n = 283)	Milk/milk and coffee /cappuccino/yogurt 115 (40.6)	Fruit juice 76 (26.9)	Tea/coffee 70 (24.7)	Chocolate 22 (7.8)
Q3: At breakfast you eat: (n = 280)	Biscuits/cakes/crackers/ breakfast cereals/bread 106 (37.9)	Fruit 17 (6.1)	Eggs and cheese 56 (20)	Dough/pizza/toast 101 (36.1)
Q4: Do you eat at least 2 portions (200g) of fruit every day? (n = 291)	Always 25 (8.6)	Often 68 (23.4)	Sometimes 129 (44.3)	Never 69 (23.7)
Q5: Do you eat at least 2 portions (200g) of vegetables every day? (n = 291)	Always 47 (16.2)	Often 77 (26.5)	Sometimes 106 (36.4)	Never 61 (21)
Q6: Do you usually eat a cake or a dessert at meals? (n = 291)	Always 79 (27.1)	Often 122 (41.9)	Sometimes 55 (18.9)	Never 35 (12)
Q7: Do you usually eat breakfast, lunch and dinner every day? (n = 290)	Always 63 (21.7)	Often 102 (35.2)	Sometimes 91 (31.4)	Never 34 (11.7)
Q8: Your diet: (n = 290)	Is different every day 157 (54.1)	Is different only sometimes during a week 88 (30.3)	Is different only during the weekend days 36 (12.4)	Is very monotonous 9 (3.1)
Q9: Your diet is based mainly on: (n = 286)	High protein content foods 35 (12.2)	High fat content foods 21 (7.3)	High carbohydrate content foods 61 (21.3)	Different foods every day 169 (59.1)
Q10: Your snacks are based mainly on: (n = 283)	Fruit/fruit juice/fruit and milk shakes/yogurt 56 (19.8)	Biscuits/crackers/bread/ stick bread 42 (14.8)	Fried potatoes/doughnuts /peanuts/soft drinks 86 (30.4)	Sweets/chocolate/ ice-cream/cakes 99 (35)
Q11: Which beverages do you usually drink between meals? (n = 287)	Water 164 (57.1)	Soft drinks 70 (24.4)	Fruit/fruit juice/fruit and milk shakes 42 (14.6)	Energy drinks 11 (3.8)
Q12: Do you drink at least 1 glass of milk or do you eat at least 1 cup of yogurt every day? (n = 289)	Always 46 (15.9)	Often 67 (23.2)	Sometimes 97 (33.6)	Never 79 (27.3)
Q13: Do you drink at least 1–1.5 L of water every day (≈4-6 cups)? (n = 289)	Always 79 (27.3)	Often 84 (29.1)	Sometimes 98 (33.9)	Never 28 (9.7)
Physical Activity and Lifestyle				
Q1: Do you usually practice a physical activity? (n = 289)	Always during the entire year 63 (21.8)	Only in some seasons 61 (21.1)	Sometimes 125 (43.3)	Never 40 (13.8)
Q2: How many hours do you practice it? (n = 288)	None 108 (37.5)	1h–2h in a week 122 (42.4)	3h–4h /week 32 (11.1)	More than 4h / week 26 (9)

Physical Activity and Lifestyle				
Q3: What do you prefer to do during free time? (n = 288)	Walking 25 (8.7)	Watching TV/listening to music /using the computer /reading a book 212 (73.6)	Practicing a sport 23 (8)	Shopping 28 (9.7)
Q4: How many hours do you spend on the computer or watching TV? (n = 288)	1h–2h a day 74 (25.7)	3h–4h a day 94 (32.6)	5h–6h a day 40 (13.9)	More than 6h a day 80 (27.8)
Q5: Your lifestyle is: (n = 289)	Very sedentary 16 (5.5)	Sedentary 91 (31.5)	Moderately active 144 (49.8)	Very active 38 (13.1)

[1] *Values expressed as n (%)*

Table 3. Differences in nutrition related variables among study locations in Saudi Arabia (Riyadh and Al-Khobar)

Variable (n)	Scores	Riyadh, n = 160	Al-Khobar, n = 131	P-Value
Eating habits [1] (n= 255)	21.7 ± 5.0 [a]	21.1 ± 4.9, n = 139 [b]	22.5 ± 5.1, n = 116	0.028 *
Physical activity and lifestyle [2] (n = 283)	6.2 ± 2.9	5.7 ±2.9, n = 154	6.8 ± 2.9, n = 129	0.002 *
Self-efficacy [3] (n = 269)	13 ±2.7	12.8 ± 2.8, n = 148	13.4 ± 2.7, n = 121	0.049 *
Awareness of healthy dietary habits [4] (n = 268)	2.6 ±1.6	2.6 ± 1.6, n = 152	2.6 ± 1.6, n = 116	0.668
Nutritional knowledge [5] (n = 252)	4.9 ± 1.9	4.7 ± 1.8, n = 143	5.1 ± 2.0, n = 109	0.190

Independent T-test.
** Significant findings.*
a Mean score ±standard deviation.
b Values are expressed as mean ± standard deviation,n.
1 Total score for eating habits section = 39.
2 Total score for physical activity and lifestyle section = 15.
3 Total score for self-efficacy section = 16. Non-parametric variable, Mann-Whitney U test was used.
4 Total score for awareness of healthy dietary habits section = 8. Higher score indicates lower knowledge in modifying dietary habits
5 Total score for nutritional knowledge section = 11.

Table 4. Differences in nutrition related variables among three BMI groups (underweight, normal, and overweight vs. obese)

Variable	Underweight	Normal weight	Overweight vs. obese	P-Value
Eating habits	21.6 ± 5.2, n = 67 [1]	21.5 ± 5.1, n = 158	23.2 ±5.0, n = 30	0.245
Physical activity and lifestyle	6.1 ± 3.0, n= 82	6.3 ± 3.0, n = 170	6.0 ± 2.7, n = 31	0.753
Self-efficacy [2]	13.1 ± 2.6, n = 68	13.1 ± 2.7, n = 169	12.8 ± 3.4, n = 32	0.994
Awareness of healthy dietary habits [3]	2.7 ± 1.7, n = 72	2.6 ± 1.6, n = 165	2.3 ± 1.5, n = 31	0.427
Nutritional knowledge	4.5 ± 2.0, n = 67	4.9 ± 1.9, n = 153	5.4 ± 1.7, n= 32	0.117

One-way ANOVA, post-hoc tests (Tukey's test).

[1] Values are expressed as mean ± SD, n.

[2] Non-parametric variable, Kruskal-Wallis test was used.

[3] Higher scores indicate lower knowledge in modifying dietary habits

Table 5. Variables tested for association with eating habits

Variable	Beta[1]	P-Value
Location	1.385	0.028 *
Self-efficacy	0.450	< 0.001 *
Awareness of healthy dietary habits [2]	- 0.529	0.008 *

Linear regression model
** Significant findings.*
[1] B unstandardized coefficients
[2] Higher scores indicate lower knowledge in modifying dietary habits.

Developing nutrition intervention programs must be initiated by identifying the most predictive variables of adolescent eating behaviors. The process of identifying predictive factors will assist in creating an effective framework for planning interventions. These interventions should be directed to improve predictive factors in order to improve the eating behavior. Programs that focus on the benefits of healthful foods by emphasizing the good quality and taste may be successful. Providing some convenient ways to include healthy meals and snacks has the potential to improve self-efficacy and change the eating behavior.

5. CONCLUSION

Unhealthy dietary patterns and sedentary lifestyles among Saudi adolescents are a major public concern. Particularly, female adolescents are at greater risk for physical inactivity and sedentary behaviors. Findings from this study confirm unhealthy lifestyle behaviors among female adolescents living in urbanized areas. There is an urgent need to develop nutrition awareness programs for children and adolescents to promote better dietary patterns and improve overall health. Appropriate physical activity programs should be added to female class curriculum to increase fitness among females. Additionally, health education classes and campaigns regarding nutrition and healthy food choices should be included to encourage healthier lifestyles. Future research should address these factors with a larger sample size. Assessing socioeconomic status and income for adolescent females could possibly uncover further associations between dietary consumption and weight. Initiating interventional programs to modify unhealthy dietary and lifestyle patterns among children and adolescents is highly suggested. Globally, nutrition and health professionals should focus on adolescent eating habits and tailor suitable educational strategies to improve adolescent nutrition. Providing nutrition knowledge in schools nationwide has the opportunity to develop a healthier generation.

6. STRENGTHS AND LIMITATIONS

Studies on Saudi population nutrition and dietary habits are limited. Several nutrition and lifestyle factors were tested in this study, so results can add additional insight to eating habits and lifestyle patterns of the Saudi population. This study can be used as a reference, especially for self-efficacy and nutrition knowledge among Saudi females. Selecting subjects from two different locations in Saudi Arabia has the advantage of generating a better representation of the female Saudi population. Major significant locational differences can be used as a background for future research.

One of the limitations of this study was that the information gathered was from self-reporting, including weight and height, which could limit the reliability of the results. Low rates of overweight and obese participants could be due to sample self-selection, as overweight/obese students were less likely to respond. The cross-sectional design can be considered as another limitation. Additionally, the small sample size may not be representative of the population, and some associations or significant variations might not be discovered because of that. Furthermore, dietary information provided by the female adolescents lacked quantity estimation as the focus was on the frequency of consumption rather than the amount or portion size. This could influence the results related to dietary patterns and correlations with other factors. Moreover, respondents' biases might have occurred due to survey translation.

CONSENT

As per international standard or university standard, Parental written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

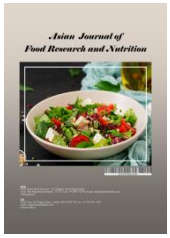
REFERENCES

1. World Health Organization. Facts Sheets of Overweight and Obesity. Available:http://www.who.int/mediacentre/factsheets/fs311/en/. March 2013.
2. Maziak W, Ward KD, Stockton MB. Childhood obesity: are we missing the big picture? Obes Rev. 2008;9(1):35-42.
3. El-Hazmi MA, Warsy AS. The prevalence of obesity and overweight in 1-18-year-old Saudi children. Ann Saudi Med. 2002;22(5-6):303-7.
4. Abalkhail B. Overweight and obesity among Saudi Arabian children and adolescents between 1994 and 2000. East Mediterr Health J. 2002;8(4-5:470-9.
5. El Mouzan MI, Foster PJ, Al Herbish AS, Al Salloum AA, Al Omer AA, Qurachi MM, Kecojevic T. Prevalence of overweight and obesity in Saudi children and adolescents. Ann Saudi Med. 2010;30(3):203 8.
6. Fleming-Moran M, Thiagarajah K. Behavioral interventions and the role of television in the growing epidemic of adolescent obesity-data from the 2001 Youth Risk Behavioral Survey. Methods Inf Med. 2005;44(2):303-9.
7. Croezen S, Visscher TL, Ter Bogt NC, Veling ML, Haveman-Nies A. Skipping breakfast, alcohol consumption and physical inactivity as risk factors for overweight and obesity in adolescents: results of the E-MOVO project. Eur J Clin Nutr. 2009;63(3):405-12.
8. Brown CM, Dulloo AG, Montani JP. Sugary drinks in the pathogenesis of obesity and cardiovascular diseases. Int J Obes (Lond). 2008;32(Suppl 6):S28-34.
9. Iannotti RJ, Wang J. Patterns of physical activity, sedentary behavior, and diet in U.S. adolescents. J Adolesc Health. 2013;53(2):280-6.
10. Al-Rethaiaa AS, Fahmy AE, Al-Shwaiyat NM.Obesity and eating habits among college students in Saudi Arabia: a cross sectional study. Nutr J. 2010;9:39.
11. Amin TT, Al-Sultan AI, Ali A. Overweight and obesity and their relation to dietary habits and socio-demographic characteristics among male primary school children in Al-Hassa, Kingdom of Saudi Arabia. Eur J Nutr. 2008;47(6):310-8.
12. Al-Hazzaa HM, Abahussain NA, Al-Sobayel HI, Qahwaji DM, Musaiger AO. Physical activity, sedentary behaviors and dietary habits among Saudi adolescents relative to age, gender and region. Int J Behav Nutr Phys Act. 2011;8:140.
13. Troiano RP, Flegal KM. Overweight children and adolescents: description, epidemiology, and demographics. Pediatrics. 1998;101(3 Pt 2):497-504.
14. Goran MI, Treuth MS. Energy expenditure, physical activity, and obesity in children. Pediatr Clin North Am. 2001;48(4):931-53.
15. Robinson TN, Killen J. Obesity prevention for children and adolescents. In: Thompson J, Smolak L, eds. Body image, eating disorders and obesity in youth: assessment, prevention and treatment. 2001;261-292.
16. Al-Hazzaa H, Sulaiman M, Al-Mobaireek K, Al-Attass O. Prevalence of coronary artery disease risk factors in Saudi children. Journal of the Saudi Heart Association 1993;5:126-133.
17. Musaiger A: Food Consumption Patterns in Eastern Mediterranean Countries. Manamah: Arab Center for Nutrition; 2011.
18. Al-Hazzaa HM: Physical activity, fitness and fatness among Saudi children and adolescents: implications for cardiovascular health. Saudi Med J. 2002;23:144-150.
19. Melnyk BM, Small L, Morrison-Beedy D, Strasser A, Spath L, Kreipe R, Crean H, Jacobson D, Kelly S, O'Haver J. The cope healthy lifestyles teen program: Feasibility, preliminary efficacy, & lessons learned from an after school group intervention with overweight adolescents. J Pediatr Health Care. 2007;21(5):315-22.
20. Horikawa C, Kodama S, Yachi Y, Heianza Y, Hirasawa R, Ibe Y, Saito K, Shimano H, Yamada N, Sone H. Skipping breakfast and prevalence of overweight and obesity in Asian and Pacific regions: a meta-analysis. Prev Med. 2011;53(4-5):260-7.
21. Musaiger A: Overweight and Obesity in the Arab Countries: the Need for Action. Bahrain Center for Studies and Research. Bahrain; 2007.
22. Al-Nuaim AA, Al-Nakeeb Y, Lyons M, Al-Hazzaa HM, Nevill A, Collins P, Duncan MJ. The Prevalence of Physical Activity and Sedentary Behaviours Relative to Obesity among Adolescents from Al-Ahsa, Saudi Arabia: Ruralversus Urban Variations. J Nutr Metab. 2012;2012: 417589.
23. Banduras A, Self-efficacy: Toward a unifying theory of behavioral change. Psychological Review. 1977;84(2):191-215

24. Ajzen, I. The theory of planned behavior. Organizational Behavior and Human Decision Processes. 1991;50:179-211.
25. Prochaska JO, Norcross JC, Fowler J, Follick MJ, Abrams DB. Attendance and outcome in a worksite weight control program: Processes and stages of change as process and predictor variables. Addictive Behaviors. 1992;17:35-45.
26. Schwarzer R. Self-efficacy in the adoption and maintenance of health behaviors: Theoretical approaches and a new model. In R. Schwarzer (Ed.), Self-efficacy: Thought control of action. Washington, DC: Hemisphere. 1992;217-243.
27. Schwarzer, R, Luszczynska, A. Perceived Self-Efficacy. Health Behavior Constructs: Theory, Measurement, and Research; 2008.
Available:http://cancercontrol.cancer.gov/brp/constructs/self-efficacy/self-efficacy.pdf
28. Hussein RA. Can knowledge alone predict vegetable and fruit consumption among adolescents? A transtheoretical model perspective. J Egypt Public Health Assoc. 2011;86(5-6):95-103.
29. Oldewage-Theron, Wilna, H., and Abdulkadir Egal. Impact Of Nutrition Education On Nutrition Knowledge Of Public School Educators In South Africa: A Pilot Study. Health SA Gesondheid. 2012;17(1):1-8.
30. Wardle J, Parmenter K, Waller J. Nutrition knowledge and food intake. Appetite. 2000;34(3):269-75.
31. Al-Almaie S. Knowledge of healthy diets among adolescents in eastern Saudi Arabia. Ann Saudi Med. 2005;25(4):294-8.
32. Al-shoshan AA. Some sociodemographic factors influencing the nutritional awareness of the Saudi teens and adults: Preliminary observations. J R Soc Health. 1990;110(6):213-6.
33. Miller, David. Saudi Arabia opens world's largest women's university. Retrieved 17 January 2012.
34. Ouda Omar K M, Towards Assessment of Saudi Arabia Public Awareness of Water Shortage Problem, Resources and Environment. 2013;3(1):10-13.
35. Turconi Giovanna, Marianna Guarcello, MD, Laura Maccarini, PhD, Federica Cignoli, MD, Stefania Setti, MD, Rosella Bazzano, Carla Roggi. Eating Habits and Behaviors, Physical Activity, Nutritional and Food Safety Knowledge and Beliefs in an Adolescent Italian Population. Journal of the American College of Nutrition. 2008;27(1)31–43.
36. Turconi G, Celsa M, Rezzani C, Biino G, Sartirana MA, Roggi C: Reliability of dietary questionnaire on food habits, eating behaviours and nutritional knowledge of adolescents. Eur J Clin Nutr. 2003;57:753–763.
37. Center for Disease Control and Prevention. Children's BMI Tool for Schools. Page last reviewed: November 30; 2011.
Available:http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/tool_for_schools.html
38. Abalkhail B, Shawky S. Prevalence of daily breakfast intake, iron deficiency anaemia and awareness of being anaemic among Saudi school students. Int J Food Sci Nutr. 2002;53(6):519-28.
39. Al-Sudairy A, Howard K. Dietary habits of technical and vocational students in Riyadh, Saudi Arabia--I. Meal skipping. J R Soc Health. 1992;112(5):217-8.
40. Farghaly NF, Ghazali BM, Al-Wabel HM, Sadek AA, Abbag FI. Life style and nutrition and their impact on health of Saudi school students in Abha, Southwestern region of Saudi Arabia. Saudi Med J. 2007;28(3):415-21.
41. Musaiger AO. Food composition tables for the Arab gulf countries. Bahrain, Arab Center for Nutrition; 2006.
42. Eaton DK, Kann L, Kinchen S, Shanklin S, Ross J, Hawkins J, Harris WA,Lowry R, McManus T, Chyen D, Lim C, Whittle L, Brener ND, Wechsler H: Centers for Disease Control and Prevention (CDC): Youth risk behavior surveillance - United States, 2009. MMWR Surveill Summ. 2010;59(5):1-142.
43. Khatib O. Non-communicable diseases: Risk factors and regional strategies for prevention and care. East Mediterr Health J. 2004;10(6):778-88.
44. Al-Hazzaa HM, Al-Nakeeb Y, Duncan MJ, Al-Sobayel HI, Abahussain NA, Musaiger AO, Lyons M, Collins P, Nevill A. A Cross-Cultural Comparison of Health Behaviors between Saudi and British Adolescents Living in Urban Areas: Gender by Country Analyses. Int J Environ Res Public Health. 2013;10(12):6701-20.
45. Taveras EM, Field AE, Berkey CS, Rifas-Shiman SL, Frazier AL, Colditz GA, Gillman MW: Longitudinal relationship

between television viewing and leisure-time physical activity during adolescence. Pediatrics. 2007;119(2): 314-319.
46. Holli BB, Calabrese RJ, Maillet JO. Communication and education skills for dietetics professionals. Philadelphia, PA: Lippincott Williams & Wilkins. 4th ed. New York: Lippincott Williams & Wilkins; 2003.
47. Rod K Dishman, Robert W Motl, Ruth Saunders, Gwen Felton, Dianne S Ward, Marsha Dowda, Russell R Pate. Self-efficacy partially mediates the effect of a school-based physical-activity intervention among adolescent girls. Preventive Medicine. 2004;38(5):628-636.
48. Glasofer DR, Haaga DA, Hannallah L, Field SE, Kozlosky M, Reynolds J, Yanovski JA, Tanofsky-Kraff M. Self-efficacy beliefs and eating behavior in adolescent girls at-risk for excess weight gain and binge eating disorder. Int J Eat Disord. 2013;46(7):663-8.
49. Chilton JM, Haas BK, Gosselin KP. The effect of a wellness program on adolescent females. West J Nurs Res. 2013.
50. Adolescent Medicine Committee, Canadian Pediatric Society. Eating disorders in adolescents: Principles of diagnosis and treatment. Pediatric and Child Health. 1998;3(3):189-11.
51. Madani KAH, Al-Amoudi NS, Kumosani TA. The state of nutrition in Saudi Arabia. Nutr Health 2000;14(1):17-31.
52. Mahfouz AA, Shatoor AS, Khan MY, Daffalla AA, Mostafa OA, Hassanein MA. Nutrition, physical activity, and gender risks for adolescent obesity in Southwestern Saudi Arabia. Saudi J Gastroenterol. 2011;17(5):318-22.
53. Abahussain NA, Musaiger AO, Nicholls PJ, Stevens R. Nutritional status of adolescent girls in the eastern province of Saudi Arabia. Nutr Health. 1999;13(3):171-7.
54. Al-Dossary SS, Sarkis PE, Hassan A, Ezz El Regal M, Fouda AE. Obesity in Saudi children: a dangerous reality. East Mediterr Health J. 2010;16(9):1003-8.
55. Janssen I, Katzmarzyk PT, Boyce WF, Vereecken C, Mulvihill C, Roberts C, Currie C, Pickett W, Health Behaviour in School-Aged Children Obesity Working Group: Comparison of overweight and obesity prevalence in school-aged youth from 34 countries and their relationships with physical activity and dietary patterns. Obes Rev. 2005;6:123-132.
56. Al- Hazzaa HM, Abahussain NA, Al-Sobayel HI, Qahwaji DM, Musaiger AO. Lifestyle factors associated with overweight and obesity among Saudi adolescents. BMC Public Health. 2012; 12:354.
57. Story M, Neumark-Sztainer D, French S. Individual and environmental influences on adolescent eating behaviors. J Am Diet Assoc. 2002;102(3 Suppl):S40-51.
58. AbuSabha R, Achterberg C. Review of self-efficacy and locus of control for nutrition- and health-related behavior. J Am Diet Assoc. 1997;97(10): 1122-32.
59. Glasofer DR, Haaga DA, Hannallah L, Field SE, Kozlosky M, Reynolds J, Yanovski JA, Tanofsky-Kraff M. Self-efficacy beliefs and eating behavior in adolescent girls at-risk for excess weight gain and binge eating disorder. Int J Eat Disord. 2013;46(7):663-8.
60. Croll JK, Neumark-Sztainer D, Story M. Healthy eating: what does it mean to adolescents? J Nutr Educ. 2001;33(4): 193-8.
61. Kosendiak A, Stanikowski P, Domagała D, Gustaw W, Bronkowska M. Dietary Habits, Diet Quality, Nutrition Knowledge, and Associations with Physical Activity in Polish Prisoners: A Pilot Study. International Journal of Environmental Research and Public Health. 2022; 19(3):1422.
62. Bonofiglio D. Mediterranean diet and physical activity as healthy lifestyles for human health. Nutrients. 2022;14(12): 2514.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/98418



Asian Journal of Food Research and Nutrition

Volume 2, Issue 3, Page 89-95, 2023; Article no.AJFRN.99211

Functional and Proximate Composition of Sorghum Starch Complemented with Germinated Moringa Seed Flour

Zubair A. B. [a*], Maxwell Y. M. O. [a], Femi F. A. [a], Ohuoba E. U. [a], Jiya M. J. [a], Isah L. R. [b] and Owheruo J. O. [c]

[a] Department of Food Science and Technology, Federal University of Technology, P.M.B.65, Minna, Niger State, Nigeria.
[b] Department of Food and Home Science, Kogi State University Anyigba, Kogi State, Nigeria.
[c] Department of Food Science and Technology, Delta State University of Science and Technology, Ozoro, Delta State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99211

Original Research Article

Received: 25/02/2023
Accepted: 27/04/2023
Published: 05/05/2023

ABSTRACT

The effect of complementing sorghum starch with moringa seed flour on the functional and proximate composition of the blends was investigated. Sorghum grains were processed in to starch and moringa seed processed to flour. The following blends were formulated; 100:0%, 97.5:2.5%, 95:5% and 92.5:7.5% for sorghum starch and moringa seed flour respectively. The samples were analyzed for functional properties and proximate composition using standard analytical procedure. Bulk density was in the range of 0.57g/ml to 0.63g/ml, swelling index was in the range of 8.06g/ml to 9.12g/ml, water absorption capacity was in the range of 1.60g/ml to 1.75 g/ml, oil absorption capacity was in the range of 1.00 g/ml to 1.30 g/ml, least gelation concentration was in the range of

**Corresponding author: E-mail: b.zubair@futminna.edu.ng;*

8% to 10%. Moisture content was in the range of 7.2% to 8.8%, fat content ranged from 9.25% to 12.25%. Fibre content was in the range of 3.01% to 3.2%. Ash content was in the range of 0.55% to 1.00%. Protein content was in the range of 6.34% to 12.15%. Carbohydrate content was in the range of 66.70% to 72.10%. Proximate composition parameters such as the fat content, protein content, ash content, fibre content, carbohydrate content increased significantly (P≤0.05) with an increasing percentage inclusion of moringa seed flour while the moisture content showed a significant decrease (P≤0.05). The functional properties parameters varied with inclusion of moringa seed flour, bulk density increased while water and oil absorption capacities, swelling index and least gelation reduced with increasing percentage inclusion of moringa seed flour. However, it was only swelling index that showed a significant decrease (P≤0.05). The study shows that inclusion of moringa seed flour to sorghum starch significantly improved the proximate composition and functional properties of the blends.

Keywords: Bulk density; fat; fibre; protein; swelling index.

1. INTRODUCTION

Sorghum is one of the essential crops in Africa, and the fifth most important cereal crop grown in the world especially in northern part of Nigeria [1]. Nigeria is the largest producer of sorghum in West Africa and it accounted for about 70% of the total sorghum output in the region [1]. Sorghum can be processed into a variety of traditional foods including fermented and non-fermented products such as unleavened bread, porridge, cookies, cakes, cereal extracts, malted alcoholic and non-alcoholic beverages, '*tuwo*', '*akamu*', '*kunu*', *'ingera*', *'kisra*' and '*koko*' [2]. Sorghum is generally high in carbohydrate, low in quantity and quality protein that is limiting in lysine, threonine, methionine and tryptophan [3]. The germ fraction of sorghum is rich in minerals, protein and lipids as well as B-group vitamins: thiamine, niacin and riboflavin [4].

Moringa is an underutilized plant; the whole seeds can be eaten green, roasted or powdered and steamed in tea and curries [5]. Moringa seed contains nutritional profile of important minerals, as well as good source of protein, vitamins, beta-carotene, amino acids and various phenolic compounds [6]. With the aforementioned nutritional profile, blending sorghum powder with moringa seed flour will increase the nutritional potential of the final product [6]. Moringa leaf can be eaten fresh or prepared similar to spinach and it contains over three times the amount of iron and vitamin A found in spinach as well as four times the amount of calcium found in cow's milk [7]. Malnutrition in its various forms (kwashiorkor, beriberi, anemia, and scurvy) is a major factor in high rates of infant mortality, for instance, in West Africa; the choice of product to combat malnutrition must comply with certain criteria: accessibility, availability in the market, low cost, ease of preparation, general acceptance, and ease of cultivation and a product that can solves the problem in a lasting way might well be moringa [8].

Complementary foods play an important role in child growth and development because it complements both nutritional and developmental needs of the infant when breast milk alone is no longer sufficient for the child [9]. Good quality weaning food must have high nutrient, bulk density, low viscosity, and appropriate texture along with high energy, protein, micronutrient contents and consistency that allows easy consumption [10]. Serious public health challenges may occur due to deficiency in essential macronutrient and micro nutrients in infant's food leading to malnutrition like marasmus or kwashiorkor among children especially in developing country [11]. Protein energy malnutrition (PEM) is the most lethal form of malnutrition commonly prevalent during the crucial transitional phase when children are weaned from breast milk to semi solids or fully adult foods. In view of this nutritional problem, several strategies have been used to formulate weaning food through a combination of locally available food materials that complement each other in such a way as to create a new pattern of essential nutrients that provide the recommended daily allowance for infants. Hence, the need for the study.

2. MATERIALS AND METHODS

Sorghum grains and Moringa seed were purchased from Kure Ultra Modern Market, Minna, Niger State, Nigeria. All chemicals used were of analytical grade. All analyses were carried out at Food Processing Laboratory, Department of Food Science and Technology,

Federal University of Technology, Minna, Niger State.

2.1 Sample Preparation

2.1.1 Preparation of sorghum starch

Sorghum starch was prepared using the method described by Zubair and Osundahunsi (2016) with slight modification. Five hundred gram (500g) of sorghum was cleaned by winnowing to remove chaffs and other light contaminants, washed in a bucket of water during which the bad seed is floated and skimmed off. The cleaned sorghum is then soaked in a bucket containing distilled water and steeped for 48 h at room temperature. The steep water was discarded and soaked grain drained and thereafter wet-milled using a Kenwood chef grinder. The slurry was then sieved through a muslin cloth to remove the over tails which were discarded. The over tails were further washed off with 600 ml of distilled water. The slurry was allowed to stand for 48 h to settle down and to promote the fermentation process at room temperature. The souring water was decanted from the sediments and the fermented slurry obtained was collected into a muslin cloth and hand squeezed to reduce the moisture content in order to facilitate the drying process. The semi-wet slurry was dried at temperature of 60^0C for 8 h. The dried slurry is then dry milled and sieved through 250 mm aperture. The resultant starch powder was cooled, packaged in airtight low-density polyethylene bags and stored under ambient condition.

2.1.2 Preparation of germinated moringa seed flour

Moringa seeds were processed according to the method described by Ijarotimi et al. [12] with slight modification. The seeds were sorted, washed and soaked in water for 12 h after which the water was drained and the seeds were spread on perforated trays lined with wet cloth and covered with another wet cloth. The seeds were allowed to germinate (sprout) at room temperature 27 ± 2^0C for a period of 72 h. The germinated seeds were picked carefully with the sprouts, washed, dehulled, oven dried at 80^0C for 9 h using cabinet drier and dry milled. It was then sieved through 250 mm aperture and packaged in airtight containers and stored under ambient condition and formulated as shown in Table 1.

Table 1. Blend formulation of sorghum and moringa seed flour

Samples	Sorghum starch (%)	Moringa seed flour (%)
A	100.0	0
B	97.5	2.5
C	95.0	5.0
D	92.5	7.5

2.2 Sample Analyses

2.2.1 Determination of functional properties

The bulk density of the sample, water and oil absorption capacities, gelation capacity and swelling power were determined by the method described by Ijarotimi et al. [12].

2.2.2 Determination of proximate composition

Moisture content, ash content, protein content, fibre content, fat content and carbohydrate content were determined using the method described by AOAC [13].

2.3 Statistical Analysis

All experiments were carried out in triplicate and data obtained were subjected to analysis of variance (ANOVA) and the means were separated by lowest standard deviation test (SPSS version 16). Significant level was accepted at 5%.

3. RESULTS AND DISCUSSION

Result of the functional property as presented in Table 2 showed that the bulk density increased significantly ($p \leq 0.05$) with an increase in the percentage of inclusion of moringa seed flour with the sample having the highest percentage inclusion recording the highest value of 0.63 g/ml and the control sample having the lowest value of 0.57 g/ml. This could be as a result of the fact that moringa seed is rich in fibre as shown in the fibre content of the sample in Table 3. Bulk density is as the ratio of flour weight to the volume in gram per mills. The value of the bulk density is close to the value of 0.54-0.71 g/ml reported by Ijarotimi et al. [12] for a weaning food from sorghum. The value is also in line with the value range of 0.58-0.61g/ml reported by Jude-ojei et al. [14] for maize-ogi supplemented with fermented moringa seed flour. The bulk density of flour samples influenced the amount and

strength of packaging material, energy density, texture, and mouth feel [8]. Nutritionally, low bulk density promotes easy digestibility of food products, particularly among children with immature digestive system [1]. Swelling capacity of food gives an indication of increase in the volume upon absorption of water. It is an important parameter that enhances the acceptability of the final product. The Swelling index recorded a significant decrease ($p \leq 0.05$) as the percentage inclusion of moringa seed flour increases. This observed decrease in the swelling power may be as a result of disruption of hydrogen atom inherent in the seed by amylases and proteases into sugars and amino acids as reported by Oloyede et al. [15]. A flour product with high swelling capacity has comparative advantages over those with low swelling capacity as the volume of the final product with high swelling capacity is of good economic advantage [15]. No significant difference ($P \geq 0.05$) was observed in the water absorption capacity of all the samples but the sample with 2.5% inclusion of moringa seed flour showed a higher value than other samples. The result range (1.6 ml/g - 1.75 ml/g) is different from the value 4.15g/ml reported by Simwaka et al. [16] for sorghum starch. This difference could be as a result of processing condition. Water absorption capacity of the control was higher than the other samples except for sample B which has the highest. This result against what was reported by [16] in water absorption capacity of 4.15ml/g of fermented Sorghum supplemented with amaranth in effect of fermentation on physicochemical and antinutritional factors of complementary foods from millet, sorghum, pumpkin and amaranth seed flours. This could be due to the decrease in amount of carbohydrates (starch) and fibre in this flour. Water absorption index plays an important role in the food preparation as it influences other functional and sensory properties [17]. It is a critical function of protein in various food products like soups, dough and baked products [17]. The water absorption capacity of flour is useful in determining the suitability of the material in bakery purposes [18]. Lower water absorption capacity is desirable for making gruels as this will help to increase the energy density and nutrient content of infant food [18]. Oil absorption capacity is an important functional property as it is attributed to the physical entrapment of oil which is considered important as flour retainer and improves the mouth feel of food products (Jude-ojei *et al*., 2017). There was no significant difference ($P \geq 0.05$) between oil absorption capacity of the control sample and the samples with inclusion of moringa seed flour. Although the control sample and the sample with 2.5% moringa seed flour have the highest value. Flour from seeds and legumes that have oil absorption capacity of more than 6.00% have been reported to perform well in the formulation of meat extenders, bakery and weaning products (Jude-ojei *et al*., 2017). The least gelation capacity of sample with moringa seed flour was found to be significantly ($P \leq 0.05$) higher than the control sample. Least gelation concentration is an index of gelation. According to Chinma et al. [8] gels are characterized by their viscosity, plasticity and elasticity and the higher the least gelation concentration, the lower is the ability of the flour to form a stable gel. The results show that sample B and C formed a stable gel than sample A and control and such product will serves as a good binder and provide consistency in food preparation such as semi-solid beverages like kunun-zaki [8]. The result was in agreement with Jude-ojei et al. [14] that reported value of 5.67-15.33% for maize starch supplemented with fermented moringa Seed flour. However, high least gelation concentration observed in control samples are desirable as Arawande et al. [19] reported that high least gelation concentration will lead to reduction in viscosity which therefore leads to increase in nutrient density and low dietary bulk which is highly favorable for a good weaning diets.

The moisture content of the sample reduces with an increasing quantity of inclusion of moringa seed flour as shown in Table 3. This is indication that the flour blend can be stored for a longer time without spoilage. Low moisture content in complementary foods is very important to prevent nutrient losses and ensure adequate shelf life of the product as the removal of moisture generally increases concentration of nutrients and make some nutrients more available [5]. The range of the moisture content (7.20 to 8.80%) is in line with protein advisory group of United Nation that recommended that moisture content of flour should not exceed 10% in order to keep a floury product for a reasonably long time [5]. The higher moisture content observed in the control sample was probably due to some variation in processing techniques.

The fat content recorded a significantly ($P \leq 0.05$) higher values with an increase in percentage inclusion seed flour than the control sample. This might be due to the fact that moringa seed is an oil seed [20]. High fat content is nutritionally advantageous because it can increase the

energy level of a diet, however, it reduce the shelf life and stability of the food product during storage since unsaturated oils are vulnerable to oxidative rancidity [21].

There was no significant difference (P≤0.05) in the fibre content of all the samples. Although, the sample with the highest percentage of inclusion of moringa seed flour (7.5%) recorded the highest value of fibre content (3.20%). The result obtained was in line with the findings of Jimoh et al. [20] that reported a value range of 1.65% to 7.94% for some selected sorghum cultivars. The fibre contents of the samples were also in line with the recommended value of less than 5% specified by the (FSSAI, 2011). Weaning food with low fibre content is very important as this would enable children to consume food that is more nutrient-dense and to meet the daily energy and other vital nutrient requirements [12]. Children are expected to have lower dietary fiber intakes than adults, with the recommended amount proportional to body weight [12]. Possible undesirable aspects of high fiber levels in weaning foods include increased bulk and lower caloric density, irritation of the gut mucosa, and adverse effects on the efficiency of absorption of various nutrients of significance in diets with marginal nutrient content [12]. Diet high in fibre content has been reported to impair protein and mineral digestion and absorption in human [22].

The protein content of all the samples that contain moringa seed flour was significantly higher than the control sample. This might be due to the fact that moringa seed is a good source of protein as reported by Ijarotimi et al. [12]. The value obtained 6.34% to 12.15% is in the range of not more than 15% recommended by (WHO, 2011). Chinma et al. [8] reported that germination and fermentation improves the protein content and quality of food products. The improvement in protein content during germination of the seeds may be attributed to the net synthesis of enzymic protein by the germinating seeds [8]. The ash content of the control sample (0.55%) was the lowest while sample B has the highest value of (1.0%). These values are lower than the value reported by Abiodun et al. [5] who reported value range of 1.01% - 1.56% for sorghum starch. The level of ash in food is an important nutritional indicator of minerals density Abiodun et al. [*5*].

Table 2. Functional properties of sorghum and moringa seed flour

Parameters	A	B	C	D
Bulk density (g/ml)	0.57^{c}±0.02	0.61^{ab}±0.01	0.58±0.01	0.63^{a}±0.00
Swelling index (g/g)	9.12^{a}±0.04	8.43^{b}±0.11	8.25^{bc}±0.07	8.06^{c}±0.02
Water absorption capacity (ml/g)	1.70^{a}±0.28	1.75^{a}±0.3	1.60^{a}±.0.28	1.60^{a}±0.28
Oil absorption capacity (ml/g)	1.30^{a}±0.14	1.30^{a}±0.14	1.10^{a}±0.14	1.00^{a}±0.00
Least gelation (%)	10.00^{a}±0.00	9.00^{a}±0.00	8.00^{b}±0.00	8.00^{b}±0.00

Values are mean ± standard deviation of duplicate determination. Mean in the same row followed by different superscript are significantly different (P≤0.05). A= control (100% sorghum starch), B= 97.5% sorghum starch and 2.5% moringa seed flour, C= 95% sorghum starch and 5% moringa seed flour, D = 92.5% sorghum starch and 7.5% moringa seed flour

Table 3. Proximate composition of sorghum and moringa seed flour

Parameters (%)	A	B	C	D
Moisture content	8.80^{a}±0.00	7.50^{b}±0.14	7.30^{bc}±0.14	7.20^{c}±0.00
Fat content	9.25^{b}±1.06	10.25^{b}±0.35	10.75^{ab}±0.35	12.25^{a}±0.35
Fibre content	3.01^{a}±0.08	3.05^{a}±0.06	3.16^{a}±0.85	3.20^{a}±0.11
Protein content	6.34^{d}±0.20	8.70^{c}±0.07	11.28^{b}±0.14	12.15^{a}±0.14
Ash content	0.55^{c}±0.07	1.00^{a}±0.00	0.63^{bc}±0.42	0.69^{c}±0.01
Carbohydrate content	72.10^{a}±0.75	67.64^{b}±0.57	66.76^{b}±0.36	66.70^{b}±0.60

Values are mean ± standard deviation of duplicate determination. Mean in the same row followed by different superscript are significantly different (P≤0.05). A= control (100% sorghum starch), B= 97.5% sorghum starch and 2.5% moringa seed flour, C= 95% sorghum starch and 5% moringa seed flour, D = 92.5% sorghum starch and 7.5% moringa seed flour

The carbohydrates content value range of 66.70%-72.10% agrees with the result 65.15%-76.28% reported by Jimoh et al. [20] for sorghum starch and also fall within the value range of 68.81%-69.65 as reported by [5]. Carbohydrate contributes to the bulk of energy of the sample which makes it high energy food and ideal for the growth of growing infants [20]. The calories in an infant diet are provided by the protein, fat and carbohydrate which are major components of complementary foods that help to meet the energy requirement of growing infants and insufficient level of any of these may lead to malnutrition [20,23,24].

4. CONCLUSION

The study showed that the addition of moringa seed flour to sorghum starch improve the functionality as well as some nutritional characteristics as shown in the protein, fat, ash and fibre content of the sample. Increases in the protein and fat content will help in reducing the incidence of protein energy malnutrition. The moisture content was also found to reduce significantly with addition of moringa seed flour hence, improvement in shelf life stability. Addition of moringa seed flour to staple food is hereby recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zubair AB, Osundahunsi OF. Effect of steeping period on the physicochemical and pasting properties of sorghum starch. Applied Tropical Agriculture. 2016;21(3):12-18.
2. Ajeigbe HA, Akinseye FM, Jonah J, Kunihya A. Sorghum yield and water Use under phosphorus fertilization applications in the Sudan Savanna of Nigeria. Global Advance Research Journal of Agricultural Science. 2018;7(8):245–257.
3. Sani RM, Haruna R, Sirajo S. Economics of sorghum (*Sorghum bicolor* (L) Moench) production in Bauchi Local Government Area of Bauchi State Nigeria. 4th International Conference of the African Association of Agricultural Economists Hammamet Tunisia. 2013;309-2016-5156.
4. Mrema E, Shimelis H Laing M, Mwadzingeni L. Integrated management of 'striga hermonthica' and '*S. asiatica'* in sorghum: A Review. Aust J Crop Science. 2020;14(1):36–45.
5. Abiodun OA, Adegbite JA, Omolola AO. Chemical and physicochemical properties of Moringa flours and oil. Global Journal of Science Frontier Research. 2012;12(1): 13–17.
6. Anjorin T.S, Ikokoh P, Okolo S. Mineral composition of *Moringa oleifera* leaves pods and seeds from two regions in Abuja Nigeria. International Journal Agriculture and Biology. 2010;12:431-434.
7. Adejumo BA, Alakowe AT, Obi DE. Effect of heat treat- ment on the characteristics and oil yield of *Moringa oleifera* seeds. The International Journal of Engineering and Science. 2013;2:232–239.
8. Chinma CE, Gbadamosi KB, Ogunsina BS, Oloyede OO, Salami SO. Effect of addition of germinated moringa seed flour on the quality attributes of wheat- based cake. Journal of Food Processing and Preservation. 2014;38:1737–1742.
9. Temesgen M. Nutritional status of Ethiopian weaning and complementary foods: A review. Scientific Report. 2013;2:1-9..
10. Singh. Recent approaches in diagnosis and control of mycobacterial infections advances in animal and veterinary sciences. 2014;2(1S):1 – 12 Special Issue – 1 (Infectious Diseases of Animals and Global Health)
11. Ayo JA, Oluwalana IB, Idowu MA, Ikuomola DS, Ayo VA. Production and evaluation of millet-egg-soybean hull composite flour: A weaning. Journal Food Nutrition. 2011;1(1):7- 13.
12. Ijarotimi OS, Adeoti OA, Ariyo O. Comparative study on nutrient composition phytochemical and functional characteristics of raw germinated and fermented *Moringa oleifera* seed flour. Food Science Nutrition. 2013;1(6):452-463.
13. Association of Official Analytical Chemist (AOAC)**.** The Official Method of Analysis 21st ed Washington DC USA; 2019.
14. Jude-Ojei BS, Ajala 'Lola Ajayi IO, Emmanuel OA, Oni SS. Physicochemical and Sensory Qualities of Maize-Ogi Supplemented with Fermented *Moringa oleifera* Seed. International Journal of Scientific Engineering and Applied Science (IJSEAS). 2017;3 (7):7-16.

15. Oloyede OO, James S, Ocheme OB, Chinma CE, Akpa VE. Effects of fermentation time on the functional and pasting properties of defatted *Moringa oleifera* seed flour. Food Sciences and Nutrition. 2015;4(1):89–95.
16. Simwaka J.E, Chamba MVM, Huiming Z, Masamba KG, Luo Y. Effect of fermentation on physicochemical and antinutritional factors of complementary foods from millet sorghum pumpkin and amaranth seed flours. International Food Research Journal. 2017;24(5):1869-1879.
17. Sreerama YN, Sashikala VB, Pratape VM, Singh V. Nutrients and anti nutrients in cowpeas and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chemistry. 2012;131(2):462-468.
18. Singh A, Yadav N, Sharma S. Effect of fermentation on physicochemical properties and invitro starch and protein digestibilities of selected cereals. International Journal of Agricultural and Food Science. 2012;2(3):66-70.
19. Arawande JO, Borokini FB. Comparative study on chemical composition and functional \ properties of three Nigerian legumes (Jack beans pigeon pea and cowpea). Journal Emerging Trends in Engineering Applied Sciences (JETEAS). 2010;1(1):89-95.
20. Jimoh WLO, Abdullahi MS. Proximate analysis of selected sorghum cultivars. Journal of Pure and Applied Sciences. 2017;10(1):285 – 288.
21. Adebayo AO, Olatodoye OP, Ogundipe OO, Akande E, Isiah CG. Production and quality evaluation of complementary foods formulated from fermented sorghum walnut and ginger. Journal Applied Bioscience. 2012;54:3901-3910.
22. Bean BW, Baumhardt RL, McCollum FT, McCuistion KC. Comparison of sorghum classes for grain and forage yield and forage nutritive value. Field Crops Research. 2013;142:20–26.
23. Al-Juhaimi F, Ghafoor K, Hawashin MD, Alsawmahi ON, Babiker EE. Effects of different levels of Moringa (*Moringa oleifera*) seed flour on quality attributes of beef burgers. CyTA - Journal of Food. 2015;14:1–9.
24. Inyang CU, Zakari KK. Effect of germination and fermentation of pearl millet on the proximate chemical and sensory properties of instant “Fura” a Nigerian cereal food. Pakistan Journal of Nutrition. 2008;7(1):9-12.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/99211