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Prevalence of Bovine Trypanosomiasis in Tembaro District, Kembata Tembaro Zone, Southern Ethiopia

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Abstract- A cross-sectional study of bovine Trypanosomiasis was conducted in Tembaro district of Kembata tembaro zone, Southern Ethiopia. A total of 800 cattle, 400 in the dry and 400 in the wet seasons were randomly selected and examined for Trypanosomiasis. The overall prevalence of the disease was 10.9%. *T. congolense* and *T. vivax* were detected from buffy coat positive samples. Among the total of 87 trypanosome infections detected in both seasons, 79.3% (69) were due to *T. congolense*; 18.4 % (16) were due to *T. vivax* and the rest 2.5% were mixed infections. Age and season of the year were not significantly associated ($P > 0.05$) with the disease. A significant association of the disease was observed ($P < 0.05$) between body condition score and sex. The packed cell volume of trypanosome infected cattle was lower compared with non-infected. According to this study, Bovine Trypanosomiasis was the major disease of cattle in the study area. Therefore control of bovine Trypanosomiasis should be enhanced systematically to improve livestock production in the area.

Keywords: bovine trypanosomiasis, prevalence, tembaro, southern ethiopia.

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Prevalence of Bovine Trypanosomiases in Tembaro District, Kembata Tembaro Zone, Southern Ethiopia

Fitsum Tessema ^α, Addisu Jimma ^ο & Amelmal Alemayehu ^ρ

Abstract- A cross-sectional study of bovine Trypanosomiases was conducted in Tembaro district of Kembata tembaro zone, Southern Ethiopia. A total of 800 cattle, 400 in the dry and 400 in the wet seasons were randomly selected and examined for Trypanosomiases. The overall prevalence of the disease was 10.9%. *T. congolense* and *T. vivax* were detected from buffy coat positive samples. Among the total of 87 trypanosome infections detected in both seasons, 79.3% (69) were due to *T. congolense*; 18.4 % (16) were due to *T. vivax* and the rest 2.5% were mixed infections. Age and season of the year were not significantly associated ($P>0.05$) with the disease. A significant association of the disease was observed ($P<0.05$) between body condition score and sex. The packed cell volume of trypanosome infected cattle was lower compared with non-infected. According to this study, Bovine Trypanosomiases was the major disease of cattle in the study area. Therefore control of bovine Trypanosomiases should be enhanced systematically to improve livestock production in the area.

Keywords: bovine trypanosomiases, prevalence, tembaro, southern Ethiopia.

I. INTRODUCTION

Livestock is essential for nutrition, food security, and livelihood of people throughout the world (Nabarro and Wannous, 2014). But animal diseases continue to constrain livestock productivity, agricultural development, human well-being, and poverty alleviation in many regions of the developing country in a variety of ways. Bovine Trypanosomiases is among the recognized constraints to livestock production in Ethiopia since it causes a severe problem in livestock, mainly in the rural community (Tulu, 2019). The disease is transmitted by tsetse flies and is endemic in a part of sub-Saharan Africa called the tsetse fly belt, which occurs approximately between latitudes 10°N and 20-30°S (Spickler, 2018). It is caused by trypanosome species *T. congolense*, *T. vivax* and *T. brucie* spp. (Giordani *et al.*, 2016). The disease epidemiology is influenced by the distribution of the vectors, the virulence of the species, and response of the host (Urquhart *et al.*, 1996). Compared to animals kept in Trypanosomiases free areas, animals kept in areas of moderate risk of Trypanosomiases have lower calving

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rates, lower milk yields, higher rates of calf mortality, and require more frequent treatment with preventive and curative doses of trypanocidal drugs. At the herd level, Trypanosomiases reduce milk off-take, live animal off-take, and the work efficiency of oxen used for cultivation (Swallow, 1999). Therefore, the objective of the current study was to determine the prevalence of bovine Trypanosomiases and associated risk factors in Tembaro woreda, Kembata tembaro zone.

II. METHODOLOGY

a) Study area and design

A cross-sectional study was conducted from 2011 to 2013 to determine the prevalence of bovine Trypanosomiases in Tembaro district, Kembata Tembaro zone, Southern Ethiopia in three randomly selected peasant associations (PAs). It is found at 7°N latitude and 37°E longitude and an altitude of 2100 meters above sea level along the Omo belt, southern Ethiopia. It is located further to the south, bordered by the Omo river and Dawro zone in the south west, and by the Hadiya zone in the north. It has a total area of approximately 27,917 km² and has a moderate tropical climate. The mixed agricultural system is practiced whereby crop production and animal husbandry take place side by side.

b) Sampling Method and Sample Size Determination

The sampling method was simple random sampling, and the sample size was determined according to the formula given by Thrusfield (2005) ($n = 1.96^2 \times P \cdot \text{exp.} / d^2$). The sample size (n) was determined considering the expected prevalence (P. exp.) of 50% and absolute desired precision (d) of 5% at a confidence interval of 95%. As a result, a total of 800 cattle were sampled (400 in the dry and 400 in the wet season).

c) Questionnaire Survey

A questionnaire survey was conducted in three kebeles of the study area from a total of 90 farmers to assess the occurrence of the disease.

d) Parasitological and hematological methods

The Buffy coat examination (BCE), the hematocrit centrifugation (HCT) and thin blood film

methods were used according to the standard procedure described by Murray *et al.*, (1977).

e) Data Analysis

Data was entered to Microsoft excel and transferred to the SPSS software version 20.0 for analysis. Chi-square was employed to determine the association of the prevalence of Trypanosomiasis with different risk factors. P-value ≤ 0.05 was considered statistically significant.

III. RESULT

a) Questionnaire survey

There are different types of livestock in the study area. These are cattle, goats, sheep, equines, and poultry. Cattle are the most predominant livestock in the study area. 95% of the interviewed farmers ranked Trypanosomiasis as a major economically important disease of cattle followed by Anthrax and Blackleg. 99% of respondents said that Trypanosomiasis (local name 'Shulule') had got clinical signs of reduced appetite, weakness, weight loss, low milk yield, reduced drought

power, diarrhea, and tail problem. The common trypanocidal drugs used according to 90% of the respondents in the study area were Diminazene aceturate, followed by Ethidium bromide. According to the survey, 90%, 8% and 2% of sick animals in the study area were treated by owners, smugglers, and veterinary personnel, respectively. About 90% of respondents said that they use one sachet of Diminazene aceturate or one tablet of Ethidium bromide per adult animal. 86% of the interviewed farmers responded that they treat with trypanocidal drugs at least two times/month/cattle especially, in the dry season.

b) Parasitological Survey

The overall prevalence of Trypanosomiasis during the study was 10.9 % (87/800), of which 11.8% is in the dry season and 10% in the wet season (Table 1). There was no significant difference between the ages of the animal and season of the year. However, body condition and sex were found to be significantly associated with trypanosome infection ($p < 0.05$) (Table 1).

Table 1: Prevalence of Trypanosomiasis infection with different potential risk factors

| Risk factors | No examined | No positives | Percent | χ^2 | P- value |
|---------------|-------------|--------------|---------|----------|----------|
| Season | | | | | |
| Dry | 400 | 47 | 11.8 | 0.632 | 0.427 |
| Wet | 400 | 40 | 10 | | |
| Sex | | | | | |
| Male | 397 | 55 | 13.9 | 7.215 | 0.007 |
| Female | 403 | 32 | 7.9 | | |
| BC* | | | | | |
| Good | 182 | 14 | 7.7 | 202.66 | 0.000 |
| Medium | 496 | 15 | 3 | | |
| Poor | 122 | 58 | 47.5 | | |
| Age | | | | | |
| Adult | 586 | 65 | 11.1 | 0.107 | 0.744 |
| Young | 214 | 22 | 10.3 | | |

*Body condition

Among the total of 87 trypanosome infections detected in both seasons, 79.3% (69) were due to *Trypanosoma congolense*; 18.4 % (16) were due to *Trypanosoma vivax* and the rest 2.5% were mixed

infections. *Trypanosoma congolense* was the dominant species identified in both the dry and the wet seasons (Table 2). The higher number of cattle which have lower PCV were infected animals (parasitaemic)(Table 3).

Table 2: Trypanosome species in dry and wet seasons

| Season | Total sample | Positives | Tc | | Tv | | Tb | Mixed | | Prevalence (%) |
|--------|--------------|-----------|----|------|----|------|------|-------|-----|----------------|
| | | | No | (%) | No | (%) | No/% | No | (%) | |
| Dry | 400 | 47 | 39 | 83 | 7 | 14.9 | 0 | 1 | 2.1 | 11.8 |
| Wet | 400 | 40 | 30 | 75 | 9 | 22.5 | 0 | 1 | 2.5 | 10 |
| Total | 800 | 87 | 69 | 79.3 | 16 | 18.4 | 0 | 2 | 2.3 | 10.9 |

Tc=*T. congolense*, Tv= *T. vivax*, Mixed= *T. congolense* and *T. vivax*

Table 3: PCV of parasitaemic cattle

| Season | No of cattle | Parasitaemic PCV<26(%) | Parasitaemic PCV>26(%) |
|--------|--------------|------------------------|------------------------|
| Dry | 400 | 30(63.8) | 17(36.2) |
| Wet | 400 | 32(80) | 8(20) |
| Total | 800 | 62(71.3) | 25(28.7) |

IV. DISCUSSION

The overall prevalence of Trypanosomiasis, according to the present study was 10.9%. This result was higher than the result of Bizuayehu *et al.* (2012), which was 6.9% in Chena district, Keffa zone, Southern Ethiopia. The result was lower than the report of Ataro *et al.* (2016) (21.33%) in Konta Special Woreda, Southern Ethiopia.

T. congolense was the species highly detected from infected animals, and *T. vivax* was found in lower prevalence. This result is similar to the reports of Yigzaw *et al.* (2017) in Anderacha district of Sheka zone, Southern Ethiopia. But this result was different from the report of Bishaw *et al.* (2012), which revealed the majority of infections were due to *T. vivax* in Wemberma district of West Gojjam Zone, North West Ethiopia.

Even though the prevalence of Trypanosomiasis was relatively higher in the dry season than the wet season, there was no significant difference between seasons of the year ($P > 0.05$). The prevalence of trypanosomosis was higher in Adults than young ones with no statistically significant difference ($P > 0.05$) among age groups. This result agrees with a study conducted by Yigzaw *et al.* (2017) in Anderacha district of Sheka zone, Bizuayehu *et al.* (2012) in Chena Wereda, and Bishaw *et al.* (2012) at Wembera district of West Gojam. This result is different from a study conducted by Teka *et al.* (2012) at Arbaminch.

There was a significant difference between body condition score in which the high prevalence of the disease was observed in cattle with poor body condition score. It was similarly reported by Bishaw *et al.* (2012) in Wembera district of West Gojam and Yigzaw *et al.* (2017) in Anderacha district of Sheka zone.

V. CONCLUSION

The study revealed that Bovine Trypanosomiasis was the major disease of cattle in the study area. *T. congolense* was the most prevalent species compared to others. Statistically, significant difference was observed in the prevalence of Trypanosomiasis between body condition score and sex. The observed association between the infection and reduction in PCV showed the impact of the disease on the productivity of infected cattle. Further studies should be conducted, including the drug resistance pattern, and Control of bovine Trypanosomiasis should be enhanced systematically to improve livestock production in the area.

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Effect of Seedling Density on Morphological Attributes of Cabbage, Cauliflower and Broccoli under Protected Condition

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Abstract- One of the most crucial and substantial factor in any of the crop for its triumph, seedling quality remains the foremost position which can be enhanced by its density resulting in the robustness, ultimately increasing the overall performance of vegetable crop. Nevertheless, their densities give rise to the vigor one influencing their survival and early establishment, being the major consideration affecting the growth and health of seedling further influencing the crop productivity. Therefore, an experiment was carried out to identify the optimum seedling density and study their effect on different morphological attributes of Broccoli cv. Green Pia, Cabbage var. Green Top and Cauliflower var. Snow Mystique in both lab and field conditions. The research design was Randomized Complete Block Design with four treatments viz. 0.5cm × 1.0cm (T1), 1.0cm × 1.0cm (T2), 1.5cm × 1.5cm (T3), and 2.0cm × 2.0cm (T4) replicated five times. Destructive sampling was done after 23 days of seed sowing to study the growth attributes viz. plant height, number of leaf, leaf area, root length, root fresh weight, shoot fresh weight, shoot dry weight and dry weight percentage. Software image J was used to measure leaf area, MS-Excel for data tabulation which were analyzed by using GenStat 15th Ed. Germination percentage was 84%, 86%, 88.0% in lab while seedling establishment was 53%, 66.3%, 70.3% in nursery bed respectively for Cabbage, Cauliflower and Broccoli.

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Effect of Seedling Density on Morphological Attributes of Cabbage, Cauliflower and Broccoli under Protected Condition

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Abstract- One of the most crucial and substantial factor in any of the crop for its triumph, seedling quality remains the foremost position which can be enhanced by its density resulting in the robustness, ultimately increasing the overall performance of vegetable crop. Nevertheless, their densities give rise to the vigor one influencing their survival and early establishment, being the major consideration affecting the growth and health of seedling further influencing the crop productivity. Therefore, an experiment was carried out to identify the optimum seedling density and study their effect on different morphological attributes of Broccoli cv. Green Pia, Cabbage var. Green Top and Cauliflower var. Snow Mystique in both lab and field conditions. The research design was Randomized Complete Block Design with four treatments viz. 0.5cm × 1.0cm (T1), 1.0cm × 1.0cm (T2), 1.5cm × 1.5cm (T3), and 2.0cm × 2.0cm (T4) replicated five times. Destructive sampling was done after 23 days of seed sowing to study the growth attributes viz. plant height, number of leaf, leaf area, root length, root fresh weight, shoot fresh weight, shoot dry weight and dry weight percentage. Software image J was used to measure leaf area, MS-Excel for data tabulation which were analyzed by using GenStat 15th Ed. Germination percentage was 84%, 86%, 88.0% in lab while seedling establishment was 53%, 66.3%, 70.3% in nursery bed respectively for Cabbage, Cauliflower and Broccoli. Significantly longer root length (4.83 cm) was obtained in 2.0cm×2.0cm which was at par with spacing 1.5cm×1.5cm whereas shorter root length (3.62cm) was obtained in 0.5cm×1.0cm. Similar trend was obtained in number of true leaves and leaf area. Similarly, significantly higher root length (4.93 cm), shoot fresh weight (1.03 g), shoot dry weight (0.09 g), leaf number(1.91) and leaf area (14.85 cm²) were obtained in 2.0cm×2.0cm for Cauliflower whereas, in the seedlings of Broccoli root length was found significantly higher in the treatment 2.0cm × 2.0cm whereas lower in 1.0cm × 1.0cm which was at par with other treatments. Similarly, leaf area in the treatment 2.0cm × 2.0cm was significantly higher while lowest in 0.5cm × 0.5cm whereas other treatments were at par. For proper seedling production and establishment wider spacing was found superior over narrow spacing.

1. INTRODUCTION

Considering the economy, better production and productivity plays the vital role, however poor seedling quality and management practices

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constrains them. The most important factors contributing towards high productivity of any crop is quality planting material as worthiness of a farmer's effort depends on the good quality of planting material to transfer seedling in the main field (Ferguson *et al.*, 1991). Along with various climatic and edaphic conditions, seedling density is one of the important factors that determine the health of the seedlings in the nursery bed. A vegetable nursery must ensures proper establishment for raising or handling of young vegetable seedlings until they are ready for permanent planting as it was reported that 20% of total production depends on good quality seeds which can be extended up to 45% by efficient nursery management (Feijter, 2015). Plant density is an important agronomic factor and the chosen crop spacing must be based on the hypothesis that optimal PPD allows interception of all (≥95%) of the available photosynthetically active radiation (Duncan, 1986; Purcell *et al.*, 2002).

The two distinct phases in the life cycle of the plants: seed to emergent seedlings; and emergent seedlings to established plants; where former phase includes the process of emergence, with the end product being density of seedlings, and the later phase includes survival and growth rate, with the end products being density and dry weight of survivors. Increasing seed density decreased the probability of a seedling emerging, while measures of growth or survival at later life stages were unaffected by the initial seed density (Callaway & Walker 1997; Holmgren *et al.* 1997).

The present research is thus carried out to investigate the effect of seed spacing on the robustness, health and growth of seedling of members of the Cole crops *i.e.* Cabbage, Cauliflower and Broccoli as good estimation of seed rate to produce the maximum number of quality seedling is the next problem faced by the farmers. This study elucidates the best method for quality seedling production and vegetables to find out the best one for the recommendation to the extension worker, vegetable growers, seed and seedling growers, besides unemployed youth and other stakeholders who intend to make vegetable seedling production as a business and the farming community as a whole directly and indirectly.

II. MATERIALS AND METHODOLOGY

The study was carried out at Horticultural Research lab and farm of Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Tribhuvan University during the autumn season of 2018. Germination % was calculated in lab along with determination of different physical characteristics of seed viz. colour, diameter, shape, test weight and purity% whereas seedling establishment % was assessed in nursery bed. The experiment was laid out in single factor Randomized Complete Block design (RCBD) with four treatments viz. 0.5×1.0 (cm^2), 1.0×1.0 (cm^2), 1.5×1.5 (cm^2) and 2.0×2.0 (cm^2) replicated five times. Field was prepared on the month of the September with the help of spade followed by land leveling, drenching with the fungicide named SAAF which was then solarized. After the solarization of bed for three days, the seeds of the Cauliflower var. Snow Mystique, Cabbage var. Green top and Broccoli var. Green Pia was sown on the line with the same spacing mentioned in the treatment. Recommended doses of NPK and vermicompost were applied (5.34g Urea, 8.60g DAP, 4.95g MOP and 1.8 kg vermicompost for the area of 0.99m^2) as a source of fertilizer. Cypermethrin as a plant protection was broadcasted in the bed after the fourth days of seed sowing. Mulching was done and irrigation was done whenever necessary. Germination and establishment of seedling were thoroughly observed for 12 days and after the 23rd days, destructive sampling method was carried out for further observation. Data were recorded for plant height, root length, leaf number, its area, LAI and root and shoot biomass. A software tool called ImageJ was used for assessing leaf area. ImageJ is a java based image processing program used to measure area via pixel counting of the image. Obtained data were entered using MS- Excel (2010), analyzed statistically using Genstat 15th edition at 5% level of significance and were interpreted using MS-Word (2010).

III. RESULTS AND DISCUSSIONS

a) Germination% and Seedling establishment%

The germination test revealed 84%, 86% and 88% germination of Cabbage, Cauliflower and Broccoli respectively in lab whereas seedling establishment was found to be 53%, 66.33% and 70.33% in field condition on the 12th day of seed sowing. However, the reason for the reduction of the seedling establishment in field after the germination was due to the problem of damping off and insect cutting.

b) Effect of seedling density on plant height (cm), root length (cm), leaf area (cm^2), number of leaves, fresh weight of both root and shoot (g), dry weight of shoot (g) and dry weight percentage of Cabbage var. Green Top, Cauliflower var. Snow Mystique and Broccoli c.v. Green Pia

The table below represents effect of seedling density on plant height (cm), root length (cm), leaf area (cm^2), number of leaves, fresh weight of both root and shoot (g), dry weight of shoot (g) and dry weight percentage of Cabbage, Cauliflower and Broccoli seedling.

A. Effect of seedling density on Cabbage.c.v. Green Top

i. Plant height(cm)

There was no significant effect of seedling density on plant height. Similar, result was found by (Mohamed, 2002) who reported that plant population had no significant effect on plant height on cowpea.

ii. Root length(cm)

Spacing had significant effect on the root length. $2.0\text{cm} \times 2.0\text{cm}$ produced the longest root length (4.83cm) which was statistically at par with $1.5\text{cm} \times 1.5\text{cm}$. Shortest root length (3.62 cm) was noted in $0.5\text{cm} \times 1.0\text{cm}$ which was statistically at par with $1.0\text{cm} \times 1.0\text{cm}$, $1.0\text{cm} \times 1.0\text{cm}$ and $1.5\text{cm} \times 1.5\text{cm}$ were statistically at par with each other. $2.0\text{cm} \times 2.0\text{cm}$ produced significantly superior root length to $0.5\text{cm} \times 1.0\text{cm}$ and $1.0\text{cm} \times 1.0\text{cm}$. With increasing plant density, light interception per plant decreases, resulting in a reduction in whole-plant photosynthesis and biomass accumulation. Therefore, carbon allocated to the roots can be greatly reduced and the total length of the roots is reduced under high plant density and increase in low plant density. Minami and Sirkar reveal the same results regarding root length, by increasing plant to plant spacing increases root length. The findings of Chatterjee and Som also have the similar results.

iii. Number of true leaves

Significant result was recorded for number of true leaf area. Highest no of leaf (3.20) was found in $2.0\text{cm} \times 2.0\text{cm}$ which was statistically at par with $1.5\text{cm} \times 1.5\text{cm}$. The least amount of leaf (2.61) was found in treatment $0.5\text{cm} \times 1.0\text{cm}$ which was statistically at par with $1.0\text{cm} \times 1.0\text{cm}$ and $1.5\text{cm} \times 1.5\text{cm}$. $2.0\text{cm} \times 2.0\text{cm}$ was significantly superior to $0.5\text{cm} \times 1.0\text{cm}$ and $1.0\text{cm} \times 1.0\text{cm}$. In wider spacing there is less competition for nutrients, moisture and light among the plants to achieve the required food for their growth and plants that receive more light tend to have more leaves (Milthorpe and Mourby, 1979). (Kathirvelan and Kalaiselvan, 2007) observed that there could be more feeding zone which encourages lateral growth resulting in the production of more branches and leaves

per plant. These results are in agreement with the previous findings by (Alege and Mustapha, 2007). Same results are confirmed by the findings of Shrivastava that as we increase the spacing between two plants, there will be a significant increase occur in number of leaves.

iv. Leaf area(cm^2)

There was significant effect of seedling density in leaf area. Maximum leaf area ($22.28 cm^2$) was found in the treatment $2.0cm \times 2.0cm$ which was statistically at par with $1.5cm \times 1.5cm$. Minimum leaf area ($10.28 cm^2$) was found in treatment $0.5cm \times 1.0cm$ which was statistically at par with $1.0cm \times 1.0cm$ and $1.5cm \times 1.5cm$. $2.0cm \times 2.0cm$ was superior to $0.5cm \times 1.0cm$ and $1.0cm \times 1.0cm$. Difference in leaf area by variable plant spacing might be due to the favorable environmental conditions leads to increase in leaf size. Results are in conformity with the finding of (Cebula *et al.*, 1994) in white cabbage. It lines with the finding of (singh and singh 2000) who stated that the maximum number of leaves were noted at wider spacing.

v. Fresh weight root(g)

Significant results were recorded for root fresh weight. $2.0 cm \times 2.0cm$ produced the maximum fresh weight ($0.055 g$). Minimum fresh weight ($0.025g$) was

noted in $0.5cm \times 1.0 cm$ which was statistically at par with $1.0cm \times 1.0cm$ and $1.5cm \times 1.5cm$. $2.0 cm \times 2.0cm$ was superior to $0.5cm \times 1.0 cm$, $1.0cm \times 1.0cm$ and $1.5cm \times 1.5cm$. The wider spacing provided more chance for development of root by proper utilization of assimilate which resulted in a maximum root weight (Hussain *et al.*, 2008). Similar results were obtained in onion by (Khushk *et al.*, 1990) and in radish by (Pervaz *et al.*, 2004).

vi. Shoot dry weight(g)

Significant results were recorded for the shoot dry weight. $2.0 cm \times 2.0cm$ produced the highest dry weight ($0.090g$) which was statistically at par with $1.0cm \times 1.0cm$ and $1.5cm \times 1.5cm$. Lowest dry weight ($0.035 g$) was noted in $0.5cm \times 1.0 cm$ which was statistically at par with $1.0cm \times 1.0cm$ and $1.5cm \times 1.5cm$. $1.0cm \times 1.0cm$ and $1.5cm \times 1.5cm$ were also at par with each other. Dry weight is a net result of photosynthesis activities thoroughly. Sunlight is a major factor in the photosynthesis process, the more sunlight it receives, the photosynthesis process can run well, resulting in more photosynthate made. Larger photosynthetic production will form larger plant organs which those affecting the increase of dry weight of plants.

Table 1: Effect of seedling density in morphological character of Cabbage in Lamjung in 2018

| Treatment (cm^2) | Plant height (cm) | Root length (cm) | Leaf area (cm^2) | No. of leaves | Root FW(g) | Shoot FW (g) | Shoot DW (g) | DW % |
|-------------------------|-------------------|--------------------|----------------------|--------------------|--------------------|--------------|---------------------|-------|
| 0.5×1.0 | 6.72 | 3.62 ^c | 10.28 ^b | 2.61 ^b | 0.025 ^b | 0.621 | 0.035 ^b | 9.979 |
| 1.0×1.0 | 5.89 | 3.81 ^{bc} | 12.72 ^b | 2.65 ^b | 0.036 ^b | 0.688 | 0.060 ^{ab} | 7.296 |
| 1.5×1.5 | 5.99 | 4.58 ^{ab} | 14.01 ^{ab} | 3.00 ^{ab} | 0.039 ^b | 0.804 | 0.065 ^{ab} | 7.840 |
| 2.0×2.0 | 6.27 | 4.83 ^a | 22.28 ^a | 3.20 ^a | 0.055 ^a | 1.143 | 0.090 ^a | 8.555 |
| Grand mean | 6.22 | 4.21 | 14.8 | 2.86 | 0.039 | 0.815 | 0.063 | 7.42 |
| F test($\alpha=0.05$) | NS | * | * | * | * | NS | * | NS |
| CV % | 13.5 | 15.6 | 41.1 | 11.5 | 29.5 | 37 | 37.5 | 44.4 |
| LSD | 1.15 | 0.90 | 8.40 | 0.45 | 0.0159 | 0.414 | 0.0325 | 4.539 |

NS non-significant, * significant at 5% and **highly significant at 1%.

Values within the same column with a common alphabet are not significantly different

B. Effect of seedling density on Cauliflower c.v. Snow Mystique

i. Plant height(cm)

There was no significant difference in plant height among the seedling obtained from various treatments. On contrary to this result, some of the research findings revealed the increase in plant height whereas some revealed the decrease in plant height with increment in the plant density. Qodliyatiet.*al* (2018) in his experiment in Cassava found out the increase in plant height in wider spacing. The reason he stated was that the wider planting space will increase the availability of nutrients and water for individual plants so that the growth of the plant increases. These findings are in

close conformity with the findings of Rahmanet.*al.* in cauliflower, Saikiaet.*al.* (2010) in broccoli also. However, Tejaswiniet.*al* (2018) in his experiment in broccoli found out the increase in plant height in narrow spacing. This might be due to more terminal increase than later growth and mutual shading in closer spaced plants.

ii. Root length(cm)

The effect of seedling density on root length was found to be highly significant. Longer root length ($4.928 cm$) was obtained in wider spacing of $2.0cm \times 2.0cm$. And significantly shorter root length ($3.676 cm$) was obtained in the spacing of $1.5cm \times 1.5cm$ which was found to be statistically at par with the treatments of spacing of $1.0cm \times 1.0cm$ and $0.5cm \times 1.0cm$. This

finding is in close conformity with the research of Amjad and Anzum (2001) in carrot plant. And the reason could be more availability of moisture in the case of higher spacing that cause higher proliferation of the root leading to longer root length.

iii. Number of true leaves

The effect of seedling density on number of true leaves was found to be significant. Significantly more number of true leaves (2.720) was obtained in wider spacing of 2.0cm×2.0cm which was found to be statistically at par with the treatment of 1.5cm×1.5cm and 1.0cm×1.0cm whereas significantly less number of true leaves (1.910) was obtained in spacing of 0.5cm×1.0cm which was also found to be statistically at par with the treatment of 1.0cm×1.0cm. Moniruzzaman (2011) in hybrid cabbage and also Chiluwale*et al.* (2018) in cane plant obtained the similar result in his experiment where wider spacing showed higher number of leaves. The reason for higher number of leaves in case of lower seedling density might be due to the lesser competition for nutrients and light among the plants. Enhanced light interception due to wider spacing certainly had a positive effect on leaf number. Hence in wider spacing due to availability of more space and light, the crop might have pronounced more number of leaves per plant.

iv. Leaf area(cm^2)

Result showed significant differences for the leaf area of seedlings under various treatments. Significantly larger leaf area (14.850 cm^2) was obtained in the spacing of 2.0cm×2.0cm which was at par with the spacing of 1.5cm×1.5cm and 1.0cm×1.0cm whereas significantly smaller leaf area (5.600 cm^2) was obtained in the spacing of 0.5cm×1.0cm which was also at par with the spacing of 1.5cm×1.5cm and 1.0cm×1.0cm. More number of leaf was observed in wider spacing which might be contributing for more leaf area. Strecket*et al.* (2014) found the result in close accordance with this result where minimum leaf area was observed in narrower spacing. In narrow spacing, water stress is seen, and there might be the decline in the cell enlargement resulting from reduced turgor pressure which cause the reduction in the leaf area (Shao *et al.*, 2008).

v. Shoot fresh weight(g)

Data concerning this parameter were subjected to statistical analysis and result showed significant differences for the fresh weight of shoot. Maximum fresh weight (1.030g) was obtained in spacing of 2.0cm×2.0cm and minimum fresh weight (0.400g) was found in the treatment of 0.5cm×0.5cm which was found to be statistically at par with the treatment of 1.0cm×1.0cm. Wider spacing allows for the growth of the lateral branches contributing to the higher fresh weight of shoot. More light interception will enhance the photosynthesis process in wider spacing which will

ultimately increase the fresh weight of shoot. Qodliyati and Nyoto(2018) in their research in arrowplant also found the similar result in case of arrow plant. And in case of narrow spacing reduction in fresh weight of shoot is due to decrease in photosynthesis and canopy structure during the water and nutrient stress (Bahreininejad *et al.*,2013).

vi. Root fresh weight(g)

Analysis showed non-significant result in case of fresh weight of root between the treatments. And in contrast with this result Ali *et al.* (2018) in turnip, Khusket*et al.* (1990) and Pervazet*et al.* (2004) in radish obtained the higher fresh weight of root in the case of wider spacing. The length of the root gets increased with increase in spacing which allows for the accumulation of higher photosynthate and ultimately the fresh weight of the root gets increased. And in case of narrow spacing there will be lower availability of the moisture around the root due to the competition and thus a lesser proliferation of root biomass resulting in the lower absorption of nutrients and water leading to production of lower biomass (Singh *et al.*1997).

vii. Shoot dry weight(g)

The result showed significant effect on dry weight of shoot among various treatments. Highest dry weight of shoot (0.095g) was obtained in wider spacing of 2.0cm×2.0cm which was found to be statistically at par with the spacing of 1.5cm×1.5cm and the lowest dry weight of shoot (0.031g) was found in the narrow spacing of 0.5cm×0.5cm which was statistically at par with the spacing of 1.0cm×1.0cm. The reason behind the higher dry weight of shoot in wider spacing could be the more accumulation of the photosynthate in case of the wider spacing. Dry weight is the net result of photosynthesis activities. And the major factor in the photosynthesis process is the sunlight, the more sunlight it receives, the photosynthesis process can run well, resulting in more photosynthate. And the seedling could receive more sunlight in the case of wider spacing in comparison with the narrow one. And this larger photosynthetic production will increase the dry weight of the shoot. Similar result is obtained in the research of arrow plant. (Qodliyati and Nyoto, 2018).

viii. Dry weight %

Data concerning this parameter were subjected to statistical analysis and result showed non-significant result for the dry weight %. Dry weight% depends upon both the dry and fresh weight of the plant. Actually dry weight % is the ratio of fresh weight to the dry weight multiplied by 100.

Table 2: Effect of seedling density in morphological character of Cauliflower in Lamjung in 2018

| Treatment (cm ²) | Plant height (cm) | Root length (cm) | Leaf area (cm ²) | No. of leaves | Root FW(g) | Shoot FW (g) | Shoot DW (g) | DW % |
|------------------------------|-------------------|-------------------|------------------------------|--------------------|------------|--------------------|---------------------|--------|
| 0.5 × 1.0 | 6.70 | 3.78 ^b | 5.60 ^b | 1.91 ^b | 0.017 | 0.40 ^c | 0.031 ^c | 7.57 |
| 1.0 × 1.0 | 6.74 | 3.57 ^b | 10.58 ^{ab} | 2.39 ^{ab} | 0.021 | 0.65 ^{bc} | 0.061 ^{bc} | 10.08 |
| 1.5 × 1.5 | 6.58 | 3.68 ^b | 10.84 ^{ab} | 2.44 ^a | 0.025 | 0.73 ^b | 0.066 ^{ab} | 9.06 |
| 2.0 × 2.0 | 6.29 | 4.93 ^a | 14.85 ^a | 2.72 ^a | 0.040 | 1.03 ^a | 0.095 ^a | 9.11 |
| Grand mean | 6.58 | 3.99 | 10.47 | 2.36 | 0.026 | 0.70 | 0.0631 | 8.95 |
| LSD (0.05) | 1.16 | 0.41 | 5.595 | 0.49 | 0.0172 | 0.29 | 0.0304 | 2.18 |
| Significance Level | NS | ** | * | * | NS | * | * | NS |
| CV% | 13.1% | 7.50% | 38.80% | 14.90% | 47.90% | 30.70% | 35.00% | 17.70% |

NS non-significant, * significant at 5% and **highly significant at 1%,

Values within the same column with a common alphabet are not significantly different

C. Effect of seedling density on Broccoli c.v. Green Pia

i. Plant height(cm)

It was significantly higher (9.08cm) in higher density (0.5cm×1cm) over other treatments whereas, seedlings in the spacing 1.0cm×1.0cm, 1.5cm×1.5cm and 2.0cm×2.0cm were found statistically at par in terms of its height. Seeds that are planted too close to one another are known to grow taller initially to compete for sunlight. The increase in plant height at higher PPD is probably caused through stem elongation (Reddy *et al.*, 1999; Pendersen and Lauer, 2003) and increase of number of nodes per plant (Oh *et al.*, 2007) due to mutual shading (Dominguez and Hume, 1978), while decrease of plant height above the optimal PPD is caused by inter plant competition for growth factors such as moisture, light and nutrients (Chanprasert, 1988).

ii. Root length(cm)

The effect of seedling density on root length shows that the value of the root length (5.20cm) was significantly higher in wider spacing i.e. 2.0cm × 2.0cm. Further, results in the remaining treatment were found statistically at par to each other indicating there was no any difference in choosing the spacing of 0.5cm × 1.0cm, 1.0cm × 1.0cm and 1.5cm × 1.5cm having the value 4.32cm, 4.32cm and 4.45cm respectively. A marked increase in root length was found at wider plant spacing. Bidet *et al.*, (2000) quoted that less carbohydrate in plant means shortened number, length and diameter of roots. For a given investment of carbohydrate, seedling at higher density expends more for its stem elongation to fulfill its light requirements while, seedlings with the same amount of carbohydrates utilize it for root and shoot developmental characteristics.

iii. Leaf area(cm²)

The results showed that leaf area in the wider spacing (2.0cm × 2.0cm) was significantly superior over narrow spacing having the value of 40.36cm² but significantly inferior value was obtained in the treatment 0.5cm × 1.0cm whereas, treatments 1.0cm × 1.0cm and 1.5cm × 1.5cm were significantly at par with the value 28.43cm² and 26.00cm² respectively. Leaf photosynthetic rate of plants grown under shaded conditions is lower than that of plants grown under conditions of full light, whereas the stomatal resistance to CO₂ diffusion is higher in relation to full light (Irmak *et al.*, 2008; Patakas *et al.*, 2003). Thus, lower photosynthetic activity means lower assimilates within plant leaf resulting small leaf with less leaf area.

iv. Root fresh weight(g)

The obtained result shows that highest fresh weight of root was found in 2.0cm × 2.0cm and 1.0cm × 1.0cm spacing having the value highest in former treatment (0.08g) followed by latter one (0.07g). Similarly, seedlings in treatment 0.5cm × 0.5cm cause lowest root fresh weight with the value of 0.05g. Moreover, in the treatment 1.5cm × 1.5cm, root fresh weight of the seedlings was statistically at par with the above treatments having the value of 0.06g. Root fresh weight is governed by three major factors viz. relationship between diameter and elongation attributes, topological connections of roots of different diameters and branching density of roots (Gretchen and Park, 1991). Root length is higher in plant with sparse density due to the availability of more carbohydrates needed for root development (Bidet *et al.*, 2000) while root diameter is influenced by hydraulic, nutritional constraints and temporal variations in assimilate availability (Gretchen and Park, 1991; Thaler and Pages, 1996).

v. Dry weight %

The results in the table 3, shows that dry weight % of the seedlings in the treatment 1.0cm × 1.0cm was found statistically superior to the treatments followed by 1.5cm × 1.5cm and 0.5cm × 1.0cm having the value 10.83, 10.04 and 9.25 respectively but statistically at par with 2.0cm × 2.0cm. And, treatments 1.5cm × 1.5cm and 2.0cm × 2.0cm were also found statistically at par.

The dry matter of plant material consists of all its constituents excluding water. Dry matter accumulations in the seedlings are the product of the translocation of nutrients from the cotyledons and the photosynthesis performed. For this reason, the cotyledons tend to lose dry weight during the seedling growth (Diaz- Ruiz *et al.*, 1999).

Table 3: Effect of seedling density in morphological character of Broccoli in Lamjung in 2018

| Treatment (cm ²) | Plant height (cm) | Root length (cm) | Leaf area (cm ²) | No. of leaves | Root FW(g) | Shoot FW (g) | Shoot DW (g) | DW % |
|------------------------------|-------------------|-------------------|------------------------------|---------------|---------------------|--------------|--------------|---------------------|
| 0.5 × 1.0 | 9.08 ^a | 4.32 ^b | 21.01 ^c | 2.560 | 0.048 ^b | 1.61 | 0.156 | 9.25 ^c |
| 1.0 × 1.0 | 8.03 ^b | 4.32 ^b | 28.43 ^b | 3.000 | 0.078 ^a | 1.94 | 0.209 | 10.83 ^a |
| 1.5 × 1.5 | 8.13 ^b | 4.45 ^b | 26.00 ^b | 2.840 | 0.058 ^{ab} | 1.80 | 0.180 | 10.04 ^b |
| 2.0 × 2.0 | 7.71 ^b | 5.20 ^a | 40.36 ^a | 3.080 | 0.084 ^a | 2.34 | 0.244 | 10.65 ^{ab} |
| Grand mean | 8.24 | 4.572 | 28.95 | 2.87 | 0.067 | 1.92 | 0.197 | 10.19 |
| LSD (0.05) | 0.898* | 0.4830* | 3.05** | NS | 0.025* | NS | NS | 0.740* |
| SEm± | 0.291 | 0.1567 | 0.99 | 0.1242 | 0.008 | 0.247 | 0.024 | 0.240 |
| CV% | 7.9 | 7.7 | 7.6 | 9.7 | 27.5 | 28.7 | 27.4 | 5.3 |

NS non-significant, * significant at 5% and **highly significant at 1%,

Values within the same column with a common alphabet are not significantly different

IV. CONCLUSION AND RECOMMENDATIONS

Seedlings in the spacing of 2.0cm×2.0cm performed better in respect of most of the studied morphological characteristics like: number of true leaves, leaf area and root length for production of quality seedlings in comparison with other closer spacing. And these parameters support better growth, development and establishment of the transferred seedlings. Since the trend shows better performance on decreasing the seedling density, we have to conduct further research in wider spacing to find out the optimum one before making any recommendation.

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Haematological Indices and Serum Biochemistry of Sheep Fed a Concentrate Diet Supplemented with Graded Levels of Exogenous Enzyme in the Semi-Arid

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Abstract- The study was conducted to determine the effect hematological indices and serum biochemistry of Sheep fed a rich diet supplemented with graded level of exogenous enzyme in the semi-arid region of Nigeria. Twenty Sheep of the non-descript breed used for the study. The animals were weighed, and divided into four groups. Each group of 5-animals randomly allotted to one of the 4-treatments in a Completely Randomized Design. The exogenous enzyme included in the diets at the level of 0, 200, 400, and 600g in T1, (Control), T2, T3 and T4, respectively. The result showed that PCV, Hb, WBC, and Neutrophil differed considerably ($P < 0.05$) among the treatments, while RBC, MCV, MCH, MCHC, Eosinophils and lymphocyte were not significantly, ($P > 0.05$) different between the treatments. There were significant ($P < 0.05$) differences among the treatments in blood urea, Alanine Amino Transferase and Alkaline phosphate, while creatinine, Total Protein, Albumin, Globulin and Aspartate amino Transferase were not significant ($P > 0.05$) among the treatment groups. However, the level of inclusion of an exogenous enzyme at 400g/100kg of corn cob based diets showed the best result. The utilization of the corn cob based diet treated with exogenous enzyme, had no deleterious effect on the health condition as demonstrated by the serum biochemical and hematological parameters of the Sheep.

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Haematological Indices and Serum Biochemistry of Sheep Fed a Concentrate Diet Supplemented with Graded Levels of Exogenous Enzyme in the Semi-Arid

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Abstract- The study was conducted to determine the effect hematological indices and serum biochemistry of Sheep fed a rich diet supplemented with graded level of exogenous enzyme in the semi-arid region of Nigeria. Twenty Sheep of the non-descript breed used for the study. The animals were weighed, and divided into four groups. Each group of 5-animals randomly allotted to one of the 4-treatments in a Completely Randomized Design. The exogenous enzyme included in the diets at the level of 0, 200, 400, and 600g in T1, (Control), T2, T3 and T4, respectively. The result showed that PCV, Hb, WBC, and Neutrophil differed considerably ($P < 0.05$) among the treatments, while RBC, MCV, MCH, MCHC, Eosinophils and lymphocyte were not significantly, ($P > 0.05$) different between the treatments. There were significant ($P < 0.05$) differences among the treatments in blood urea, Alanine Amino Transferase and Alkaline phosphate, while creatinine, Total Protein, Albumin, Globulin and Aspartate amino Transferase were not significant ($P > 0.05$) among the treatment groups. However, the level of inclusion of an exogenous enzyme at 400g/100kg of corn cob based diets showed the best result. The utilization of the corn cob based diet treated with exogenous enzyme, had no deleterious effect on the health condition as demonstrated by the serum biochemical and hematological parameters of the Sheep.

I. INTRODUCTION

Ruminant animals play a significant role in the agricultural economy of Nigeria. With their inherent qualities thriving under harsh environments and low capital investment by the agricultural farmer under the free-range system. Ruminants act as an insurance against crop failures and provide alternative sources of livelihood to the farmers all year round (Selvamand Safiullah, 2002).

The feed resources that provide the bulk of ruminant feed in the semi-arid zone of Nigeria include natural grasses and crop residues. These characterized by low intake and digestibility. The general awareness on the use of exogenous enzyme is due to their ability to increase the efficiency of digestion by improve the digestibility and feed energy (Anon, 2015). The

exogenous proteins, like other feed enzymes, are of natural origin and non-toxic. They are mostly commercial products of microbial fermentation which are safe, inexpensive and straightforward, substantial, reliable and agro-industrial resources (Bhat and Hazlewood, 2001)

The use of blood examination is a way of assessing the health status of animals has been documented (Muhammad *et al.*, 2000). It plays a vital role in the physiological, nutritional, and pathological state of organisms. Enzyme addition tends to increase the differential lymphocyte count (Colombatto *et al.*, 2003). The objective of this study is to determine the performance and blood indices of Sheep fed concentrate diet supplemented graded level exogenous enzymes.

II. MATERIALS AND METHODS

a) Experiment Site

The study was carried out at the Department of Animal Science Teaching and Research Livestock Farm, University of Maiduguri. Maiduguri, the capital of Borno state, is situated between latitude 11°51' North, Longitude 13° 5' East and an altitude, of 354m above sea level (DNMA, 2013). The area falls within the Sahelian region (semi-arid zone) of West Africa, which is characterized by the short duration of rainfall (3-4 months). The rainfall varies from a minimum of 478mm-500mm to maximum of 600mm-621mm (Afolayan *et al.*, 2012). The relative humidity (RH) is 32% in the morning. The minimum relative humidity (RH) is 11% in March, and the maximum is 64% in August (Afolayan *et al.*, 2012; DNMA, 2013). The mean temperature is 34°C, the maximum being 40-60°C, and the lowest 25°C, which is in April and December, respectively. The average dew point is 52%, the minimum being 32% in February, the maximum is 72% in August (Afolayan *et al.*, 2012; DNMA, 2013).

i. Animals and Experimental design

Twenty (20) Sheep of the non-descript breed weighing on average 22.65kg used. The animals obtained from the flock of Sheep kept at the Department of Animal Science Livestock Teaching and Research

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Farm, University of Maiduguri. They were weighted and identified using plastic air tags. Feeding was done at 4% body weight once daily at 8:00 am with the leftover being weighed before the next feeding. The animals divided into 4-groups, and each group of 5-animals randomly assigned to one of the treatment in a completely randomized design (CRD). The study lasted for 11 weeks.

ii. *Treatments (Experimental diets)*

The feeding ingredients used for the formulation of the experimental diets were maize cob, wheat offal, cottonseed cake, poultry litter, and exogenous enzymes. The diet formulated consisted of maize cob (40%), wheat offal (30%), cotton seed cake (15%), poultry litter (15%). The enzyme included in the diet a level of 0, 200, 400 and 600g in T1 (control), T2, T3 and T4 respectively.

Table 1: The composition of the Experimental Diets (%)

| Ingredients | Treatments and level of Enzyme supplementation (g) | | | |
|------------------|--|----------|----------|----------|
| | T1 (0) | T2 (200) | T3 (400) | T4 (600) |
| Corn cob | 40 | 40 | 40 | 40 |
| Wheat offal | 30 | 30 | 30 | 30 |
| Cotton seed cake | 15 | 15 | 15 | 15 |
| Poultry litter | 15 | 15 | 15 | 15 |
| total | 100 | 100 | 100 | 100 |

iii. *Blood Sample Collection*

a. *Blood Sample collection and analysis*

At the end of the feeding trial, three Sheep randomly selected from each treatment. The blood sample was collected from three (3) animals per treatment on the last day of the study before terminating the experiment. Blood samples were collected from each animal by jugular-venipuncture using a disposable syringe and sterile needles (18 gauge). Before feeding in the morning, bleeding done and an average of 10ml of blood was collected from each animal by jugular-venipuncture using disposable syringes and sterile needles (18 gauge). Before feeding in the morning bleeding done, and 10 ml of blood collected from each animal. The blood sample placed in two vacutainers. One contained ethylene diamine tetra-acetic acid (EDTA) for hematological studies as described by Al-Eissa and Alkahtani. (2011), the second bottles contained no, anticoagulant, and it received the remaining blood which was allowed to stand for about 2 hours at room temperature. The universal bottles there that centrifuged at 700xg for 15 minutes, the serum separated were decanted and stored in a freezer at -10C for blood the biochemical analysis as reported by Gambo et al. (2011). MCV, MCH and MCHC were deduced according to Jain (1986) as follows: $MCV (fl) = PCV \times 10 / RBC$; $MCH (\mu g) = Hb / RBC \times 10^6$; $MCHC (\%) = Hb \times 100 / PCV$.

From the centrifuged blood sample in plain bottles, serum was collected for biochemical assay. Total protein and albumin were determined by *Biuret* and *Bromocresol Green Methods*, respectively. Blood Urea Nitrogen (BUN), Creatinin, Bilirubin as well as activities of the liver enzymes (AST and ALT) were determined by Enzymatic method as outlined by Bush (1991). Burchadrection determined serum Cholesterol

b. *Statistical Analysis*

The data generated were subjected to analysis of variance (ANOVA) using the complete randomized

design (CRD), and the Duncan multiple range tests, was used for the mean separation.

III. RESULTS AND DISCUSSION

Table 2: Hematological Parameters and differential count of Sheep supplemented with graded levels of the enzyme

| Parameters | Treatments and level of Enzyme supplementation (g) | | | | SEM |
|-------------------------|--|---------------------|---------------------|--------------------|--------------------|
| | T1(0) | T2 (200) | T3 (400) | T4 (600) | |
| PCV (%) | 25 ^b | 32 ^a | 28 ^b | 28 ^b | 1.0* |
| Hb(g/dl) | 8.30 ^b | 10.53 ^a | 9.30 ^b | 9.20 ^b | 0.33* |
| RBC (×10 ⁶) | 11.67 | 13.53 | 12.22 | 10.60 | 1.01 ^{NS} |
| MCV (fl) | 22.12 | 23.65 | 22.91 | 26.42 | 2.00 ^{NS} |
| MCH (pg) | 7.11 | 7.78 | 7.62 | 8.67 | 0.80 ^{NS} |
| MCHC (%) | 33.20 | 33.91 | 33.21 | 32.85 | 7.89 ^{NS} |
| WBC (×10 ³) | 14.13 ^a | 10.67 ^{ab} | 7.60 ^b | 9.67 ^{ab} | 1.87* |
| Neutrophil (%) | 66.67 ^a | 48.67 ^b | 51.00 ^{ab} | 65.33 ^a | 4.81* |
| Lymphocyte (%) | 33.33 | 49.33 | 47.00 | 33.67 | 5.2 ^{NS} |

a,b= Means in the same row with different superscript differ significantly ($P < 0.05$)

NS= Not significant

* - Significant ($P < 0.05$).

SEM=Standard error of means

PCV=Packed Cell Volume;

Hb=Haemoglobin;

RBC=Red Blood Cell;

WBC= White Blood Cells;

MCV=Mean Corpuscular Volume;

MCH=Mean Corpuscular Haemoglobin;

MCHC=Mean Corpuscular Haemoglobin Concentration;

a) *Hematological Parameters and differential count of Sheep supplemented with graded levels of the enzyme*

The result of the hematological parameter of Sheep is present in table 1. The PCV, Hb, WBC, and Neutrophil differed significantly ($P < 0.05$) among the treatments, while RBC, MCV, MCH, MCHC, Eosinophils and lymphocyte were not significantly ($P < 0.05$) different between the treatments.

The PCV value of Sheep supplemented with varying levels of exogenous enzyme ranged from 25.0 to 32.0 %. The PCV values obtained in the study were in contrast with the value 43.8+ 0.6% reported by Egbe-Nwiyet *et al.*, (2000), while in line within the range value (27- 45%) as published by Jain. (1993) Packed cell volume is essential in the diagnosis of anemia (Chineke *et al.*, 2006). The higher PCV values obtained in this study might likely be a sign of healthier Sheep.

The hemoglobin of the Sheep ranged from 3.30 to 10.53 g/dl. The hemoglobin values were in the normal range (8-16g/dl) of hemoglobin for healthy Sheep (Greenwood, 1977).

The red blood cell count obtained in this study ranged from 10.60 to 13.53 g/dl. The values were higher than the range value (4.44 – 8.69 g/dl) reported by Njidda *et al.* (2014). Red blood cell provide information about the hemoglobin content and size of red blood cells. Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular Hemoglobin Concentration (MCHC) had showed no significant ($p >$

0.05) differences among the treatments. The values obtained for all the treatment groups indicate nutritional adequacy of all diets. Since the benefit did not indicate mal-or-under nutrition (Church *et al.* 1984). Mean corpuscular volume (MCV) values obtained ranged from 22.12 – 26.42 fl. According to Research Animal Resources (2009), the values fell within normal physiological range 23-48fl for Sheep. But reports of Borjesson *et al.* (2000) which is lower than the normal physiological MCV range for Sheep (35.3 – 43.7 fl). The value of mean corpuscular hemoglobin obtained in the study ranged from 7.11 to 8.67 pg T0 and T3 respectively, which were also lower than the values for lamb (12-20 pg) as reported by Njidda *et al.* (2014), but the study is inconformity with result 8-12 pg according to Research Animal Resources (2009). The value of MCV and MCH are significant in the diagnosis of anemia and also serve a useful index of the capacity of the bone marrow to produce red blood cells (Awodi *et al.*, 2005). Mean Corpuscular Hemoglobin Concentration obtained in this study fell within the range of 31-38% as reported by Research Animal Resources (2009). Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration low level in blood is an indication of anemia (Aster, 2004).

The white blood cell values for the Sheep ranged from 7.60 to 14.13. The white blood cell (WBC) values were within the average range values of Sheep (4-12%) reported by Research Animal Resources (2009). The higher WBCs count recorded in the Sheep not

supplemented with the enzyme may be due to the response of the animals to protect themselves against invading pathogens. This study shows that the animals were healthy because, the decrease in number of WBC below the normal range is an indicator of allergic

conditions. While elevated values (leucocytosis) indicate the existence of a recent infection, usually with bacteria (Ahamefule *et al.*, 2008). The WBCs or leucocytes are the mobile unit of the body in protecting system (Aiello, 2000).

Table 9: Effect of Graded Level of the Exogenous Enzymes in Sheep fed a concentrate diet

| Parameters | Treatments and level of Enzyme supplementation (g) | | | | SEM |
|-------------------|--|--------------------|---------------------|--------------------|--------------------|
| | T1(0) | T2 (200) | T3 (400) | T4 (600) | |
| Total protein g/l | 61.66 | 57.66 | 57.66 | 63.33 | 1.21 ^{NS} |
| Albumin g/l | 38.00 | 31.33 | 35.00 | 35.33 | 1.44 ^{NS} |
| Globulin g/l | 23.66 | 26.33 | 24.66 | 28.00 | 1.17 ^{NS} |
| Urea | 4.20 ^c | 5.53 ^b | 3.83 ^c | 6.23 ^a | 0.30* |
| Creatinine | 85.66 | 101.00 | 94.66 | 101.33 | 2.83 ^{NS} |
| ASAT | 74.66 | 62.00 | 66.66 | 69.33 | 3.95 ^{NS} |
| ALAT | 21.00 ^b | 44.66 ^a | 38.66 ^a | 41.66 ^a | 3.29* |
| ALK-phos | 124.00 ^a | 60.33 ^b | 116.66 ^a | 80.66 ^b | 8.93* |

a, b, means in the same row with different superscript differ significantly ($P < 0.05$);

NS=Not significant.

* – Significant ($P < 0.05$)

SEM – Standard error of means, AST – Aspartate Aminotransferase, ALT – Alanine Aminotransferase.

b) Serum Biochemistry Indices

The result of the serum biochemical indices of Sheep fed graded levels of the exogenous enzyme is presented in table 9. There was significant ($P > 0.05$) difference among the treatment groups in blood urea.

Total protein values were not significant ($P > 0.05$) among the treatments groups. The Total protein values ranged from 57.66 to 63.33 (g/L). The result of total protein in this study fell within the normal range (59-78g/l) as reported by Latrimer *et al.*, (2003). Albumin values were not significantly ($P > 0.05$) different among the treatment groups. The albumin values ranged from 31.33 to 38.00g/l in T2 and T1, respectively. The value recorded in this study fell within the range of 27-37g/l as reported by Latrimer *et al.*, (2003). Globulin value was not significantly different ($P > 0.05$) among the treatment groups. The globulin values ranged 23.66 to 28.00 g/dl in T1 and T4, respectively; T4 had the highest amount of globulin. This result could probably be due to the enzyme level in the various treatment groups. Globulin in the blood can be diagnostic of malfunction in the body or specific disease, such as liver disease (Anon, 1980). The values in this study fell within the average range 32-50 g/dl as reported by Latrimer *et al.*, (2003). The normal values for albumin, total protein, and globulin obtained in this study indicate nutritional adequacy of the dietary protein for utilization (Apatá 1990).

The values of urea were significantly different ($P < 0.05$) among the treatment groups. In this study, the urea values ranged from 3.83 to 6.23 mmol/l for T3 and T4, respectively. The blood urea values fell within the normal range (3.5 – 10.7mmol/l) for a healthy animal, according to Sirois, (1995). The highest value (6.23) of blood urea obtained in this study is an indication of the

inferiority of efficient utilization of nitrogen and urea recycling which might have affected the amino acid balance (Cetin *et al.*, 2009). It probably enzyme helps in the breakdown cell wall and increase amino acid content. The creatinine values were not significantly ($P > 0.05$) different among the treatment groups. Creatinine values ranged from 85-66 to 101.33 mmol/l of T1 and T4, respectively. The result of this study is in contrast with the value 62.56 reported by Njidda (2014). The result is in agreement with Latrimer *et al.*, (2003) who said the blood creatinine level of 76-174 mmol/l in Sheep. High creatinine value is indicator of inadequate protein and amino acid metabolism that can lead to impaired and cardiac infarction (Gray and Howarra, 1980). The high level of corn cob in the diet which contains lignin and probably might have had an effect on the Sheep.

Aspartate Amino Acid Transferase (ASAT) values were not significantly ($P > 0.05$) different among treatment groups. T1 (control) had the highest value of 74.66 lu/l, while T2 T3 and T4, had lower values of Aspartate Amino Transferase level in the blood. The values of Aspartate Amino Transferase fell within the average range value (40.0-123) lu/l for healthy animals (Mitruka and Rawnsley, 1977) ASAT indices is used for diagnosing hepatic damage in animals (Mohgoub *et al.*, 2008).

Alanine Amino Transferase (ALAT) in the blood were significantly different ($P < 0.05$) among the treatment groups. The ALAT values ranged from 21.00 to 44.66lu/l in T1 and T2, respectively. The values fell within the normal range of 15-44 lu/l as reported by Latrimer *et al.*, (2003). T2 had the highest value of ALAT, while the lowest value recorded in T1 (control). ALAT is a liver-specific hepatocellular enzyme used to assess liver

damage (Mahgoub *et al.*, 2008). The inclusion of the enzyme might probably affect the ALAT value of the animal.

Alkaline phosphate values were significantly different ($P < 0.05$) among the treatment groups. The alkaline phosphate values ranged from 80.66 to 124.00 lu/l in T4 and T1. T1 (control) had the highest value of alkaline phosphate, which probably might be due to the diet without enzyme. Kaneko *et al.*, (1997) reported higher values of Alkaline phosphate which were found in the 40 days of life, due to more intense bone remodeling and leakage of the enzyme from the growing bone and intestine into the blood.

IV. CONCLUSION

The utilization of the corn cob based diet treated with an exogenous enzyme. The study showed that no deleterious effect on the health conditions as determined by the serum biochemical and hematological parameters of the Sheep. However, the result showed that the level of exogenous at 400g/100kg of diet had the best result. The nutritive value of poor quality crop residue can be proved through the use of exogenous enzymes. Further research should be conducted to ascertain the level of inclusion of exogenous enzyme in ruminant diets.

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Evaluation of Different Blended Fertilizers Types and Rates for Better Production of Potato at Bule Soil Condition, Southern Ethiopia

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Keywords: macro and micronutrient, potato, sufficient nutrient, economic feasibility.

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Mulugeta Habte ^α, Atinafu Assefa ^ο & Abay Ayalew ^ρ

Abstract- Nutrient mining due to sub optimal fertilizer use in one hand and unbalanced fertilizer (only N and P) uses on other has favored the emergence of multi nutrient deficiency in Ethiopian soils. This problem demands different studies to balance the nutrient combination to improve potato yield and quality. A trial was conducted to evaluate different fertilizer types for potato production and to enrich its quality in Southern Ethiopia during the main cropping season of 2016 and 2017. Fertilizer treatments were based on limiting nutrients of the area including N, P, K, S, B and at different rate and combination. The trial consists of ten treatments (1) no fertilizer (control) (2) NPSB: 69 kg N + 23.5 kg P + 10 kg S + 1.07 kg B/ha (3) NPSB: 92 kg N + 31 kg P + 13 kg S + 1.4 kg B/ha (4) NPSB: 115 kg N + 39 kg P + 17 kg S + 1.7 kg B/ha (5) NPSB: 138 kg N + 47 kg P + 20 kg S + 2.0 kg B/ha (6) NPSBCu: 69 kg N + 31 kg P + 17 kg S + 1.4 kg B + 0.625 kg Cu/ha (7) NPSBCu: 92 kg N + 39 kg P + 10 kg S + 1.7 kg B + 0.625 kg Cu/ha (8) NPSBCu: 115 kg N + 39 kg P + 10 kg S + 1.7 kg B + 0.625 kg Cu/ha (9) NPSBCu: 138 kg N + 39 kg P + 10 kg S + 1.7 kg B + 0.625 kg Cu/ha and (10) NPS: 112 kg N + 40 kg P + 17 kg S/ha was used as positive control. In addition, except the absolute control all plots were received 60 kg K/ha. The trial was conducted on two farms and treatments were laid out in a randomized complete block design replicated three times in each farm. Crop characteristics measured were analyzed using Proc GLM procedures in the SAS 9.3 program. Economic analysis was also performed to investigate the economic feasibility of the fertilizers for potato production. Applying blended fertilizer increase potato yield. The economic analysis revealed that except treatment 2 and 3 all the treatments were dominated by the treatment with low total cost that varies. The highest net benefit was obtained from treatment 3 with acceptable marginal rate of return. However, treatment 2 also met more than the required return. This result also confirmed by the sensitivity analysis, both treatments sustains acceptable returns even under 20% input price increment. Therefore, NPSB: 69 kg N + 23.5 kg P + 10 kg S + 1.07 kg B/ha and NPSB: 92 kg N + 31 kg P + 13 kg S + 1.4 kg B/ha are recommended for potato production.

Keywords: macro and micronutrient, potato, sufficient nutrient, economic feasibility.

1. INTRODUCTION

Potato (*solanumtuberosum* L.) is the fourth most important food crop in the world after rice, maize and wheat in terms of human consumption

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(Karam et al., 2009; Kandil et al., 2011). The quantity produced yearly exceeds 300 million metric tons and more than a billion people consume worldwide. Potato is rich in carbohydrates, protein, vitamins, dietary fibers, simple sugars and minerals (CIP, 2010; FAO, 2008). However, the yield is very low (below 10 t ha⁻¹) as compared to the yield in developed countries (30 to 40 t ha⁻¹) where sufficient amount of fertilizers are applied (FAO, 1991).

Fertilizer application has important effects on the quality and yield of potatoes (Leytem and Westermann, 2005). Nitrogen supply plays an important role to balance between vegetative and reproductive growth for potato (Alva, 2004; White et al., 2007). Previous studies have shown that N fertilizer applications can increase dry matter content, protein content of potato tubers, total and/or marketable tuber yield (Zebarth et al., 2004; Zelalem et al., 2009). Nitrogen uptake on per day basis is sometime even more than 1.5 kg ha⁻¹ during active growth period (Kumar and Trehan, 2012).

Similarly, uptake of fertilizer nutrients (NPK) by potato per unit area and time is quite high because of the rapid rate of early growth and tuber bulking (Singh and Trehan, 1997). A healthy crop of potato removes about 170-230 kg K₂O ha⁻¹ indicating higher requirement for K as compared to cereals. On the other hand nutrients present in mineral fertilizers are more effective than the equivalent amount of these nutrients present in FYM (Bagdoniene et al., 1998) which indicates mineral fertilizer efficacy for potatoes was noticeably higher than that of organic fertilizer (Antanaitis and Svedas, 2000).

Nutrient mining due to sub optimal fertilizer use in one hand and unbalanced fertilizer (only N and P) uses on other has favored the emergence of multi nutrient deficiency in Ethiopian soils (Abyie et al., 2003, Beyene, 1984; Wassie et al., 2011). Currently, the soil fertility map of Ethiopia is developed by Agricultural Transformation Agency (ATA) and reported the deficient nutrients in the south nation nationalities and people regional state (SNNPRS) in 2016. Based on the soil fertility map, 13 blended fertilizers containing N, P, K, S, B, Zn and Cu in different mix form have been recommended for SNNPRS. Therefore, to benefit farmers from their small holding, identification of proper

fertilizer blends for specific site to enhance potato production is crucial.

II. MATERIALS AND METHODS

Field experiment was conducted to evaluate different blended fertilizers for potato production in Buleworeda (district) of the Southern Nations, Nationalities and Peoples Regional State (SNNPRS) in the main cropping season of 2016 and 2017. Treatments were prepared based on the nutrient deficiency of the area which indicated in the soil fertility map of Ethiopia produced by Agricultural Transformation Agency (ATA) (2016). Accordingly, three types of fertilizers (NPSB, NPSBCu and NPS) were used in different rates. The experiment consists of ten treatments (1) no fertilizer (control) (2) NPSB: 69 kg N + 23.5 kg P + 10 kg S + 1.07 kg B/ha (3) NPSB: 92 kg N + 31 kg P + 13 kg S + 1.4 kg B/ha (4) NPSB: 115 kg N + 39 kg P + 17 kg S + 1.7 kg B/ha (5) NPSB: 138 kg N + 47 kg P + 20 kg S + 2.0 kg B/ha (6) NPSBCu: 69 kg N + 31 kg P + 17 kg S + 1.4 kg B + 0.625 kg Cu/ha (7) NPSBCu: 92 kg N + 39 kg P + 10 kg S + 1.7 kg B + 0.625 kg Cu/ha (8) NPSBCu: 115 kg N + 39 kg P + 10 kg S + 1.7 kg B + 0.625 kg Cu/ha (9) NPSBCu: 138 kg N + 39 kg P + 10 kg S + 1.7 kg B + 0.625 kg Cu/ha and NPS: 112 kg N + 40 kg P + 17 kg S/ha was used as positive control. In addition, except the absolute control all plots were received 50 kg K/ha.

III. EXPERIMENTAL LAYOUT

The experiment was conducted on two farms in each year and laid out in a randomized complete block design using 3.75 m by 3.9 m plot size and replicated three times in each farm. To avoid mixing up of treatments the plots were separated by 1 and 1.5 m space between plots and blocks, respectively. All doses of NPS, NPSB and potassium fertilizers were applied at planting time and urea was top dressed 45 days after planting. Foliar application was used for copper selfate. Improved potato variety (Gudene) was planted in rows and other crop management practices were used as recommended for the crop.

IV. AGRONOMIC AND ECONOMIC ANALYSIS

Agronomic data for potato, including plant height, number of plant/hill, number of tuber/hill, above ground total biomass, marketable and unmarketable tuber yield were measured. Analysis of variance for all data was done using Proc GLM procedures in the SAS 9.3 program (SAS Institute Inc., Cary, NC USA). The least significant difference (LSD) at 5% probability level was used to establish the significance of differences between the means.

An economic analysis was used to investigate the economic feasibility of the fertilizer types (NPS, NPSB and NPSBCu) for potato production. The partial

budget, dominance and marginal rate of return were calculated. For partial budget analysis averages yield that was adjusted downwards by 10% was used, assuming that farmers would get ~10% less yield than is achieved on an experimental site. The average open market price for potato (6.5 Ethiopian Birr (ETB)/kg) and potato seed (10.0 ETB/kg); and the official prices for NPS (10.94 ETB/kg), NPSB (10.28 ETB/kg), N as Urea (8.76 ETB/kg), potassium chloride-K (14.0 ETB/kg) and copper sulfate-Cu (1000 ETB/kg) were used for the analysis. For a treatment to be considered a worthwhile option for farmers, the minimum acceptable marginal rate of return should be over 50% (CIMMYT, 1988). However, Gorfu et al. (1991) suggested a minimum acceptable rate of return should be 100%. Therefore, the minimum acceptable marginal rate of return considered in this study is 100%.

V. RESULT AND DISCUSSION

The combined analysis result presented in table 1 revealed that all plots treated with different types and rates of fertilizers significantly ($P < 0.05$) increased the marketable tuber yield and plant height of potato at Bule. In the control plot, the lowest marketable yield was measured. The yield advantage was 50.6% in the lowest yield measured from treatment 7 compared to the untreated plots (table 1). However, statistically significant difference was not observed in biomass and number of tuber per hill among all treatments. This result might be obtained due to the cumulative contribution of macro and micro nutrients which were identified as deficient soil nutrients in the soil fertility map of the area. Abay A. and Tesfaye D., 2011, reported that 111 kg N + 39 kg P ha⁻¹ or 10 t compost + 73.4 kg N + 26 kg P ha⁻¹ increased potato tuber yield. In the current study, economically feasible rates were 92 N, 31 P, 13 S, 1.4 B kg/ha. Nitrogen and phosphorus were reduced to 92 and 31 compared to the above authors. This result might be contributed from the additional micro nutrients.

Table 1: Yield and yield components of potato influenced by different blended fertilizers at Bule

| Treatments | Plant height (cm) | No. of Plant/hill | No. of tuber/hill | Unmarketable yield t/ha | Marketable yield t/ha |
|--|-------------------|-------------------|-------------------|-------------------------|-----------------------|
| 1. Control (no fertilizer) | 66.73e | 3.592 | 8.467 | 0.5 | 19.892 b |
| 2. NPSB: 69 + 23.5 + 10 + 1.07 kg/ha | 84.48d | 3.892 | 9.533 | 0.508 | 32.125 a |
| 3. NPSB: 92, 31, 13, 1.4 kg/ha | 87.91bcd | 3.875 | 9.9 | 0.533 | 34.075 a |
| 4. NPSB: 115, 39, 17, 1.7 kg/ha | 91.16bc | 3.925 | 10.33 | 0.742 | 32.817 a |
| 5. NPSB: 138, 47, 20, 2.0 kg/ha | 97.83a | 3.733 | 9.467 | 0.758 | 33.975 a |
| 6. NPSBCu: 69, 31, 17, 1.4, 0.625 kg/ha | 84.67cd | 3.492 | 8.642 | 0.592 | 30.883 a |
| 7. NPSBCu: 92, 39, 10, 1.7, 0.625 kg/ha | 87.08cd | 3.492 | 10.29 | 0.908 | 29.950 a |
| 8. NPSBCu: 115, 39, 10, 1.7, 0.625 kg/ha | 94.27ab | 4.167 | 10.4 | 0.45 | 33.392 a |
| 9. NPSBCu: 138, 39, 10, 1.7, 0.625 kg/ha | 90.73bcd | 3.708 | 9.308 | 0.625 | 32.867 a |
| 10. NPS: 112, 40, 17 kg/ha | 88.56bcd | 3.967 | 10 | 0.508 | 31.400 a |
| LSD (0.05) | 6.59 | NS | NS | NS | 5.7647 |
| CV (%) | 8.74 | 22.00 | 24.59 | 61.40 | 15.58 |

Note: Values followed by the same letter are not significantly different at $P < 0.05$.

VI. ECONOMIC ANALYSIS

The dominance analysis (table 2) showed that except treatment 2 and 3 all other treatments were dominated by the treatments with lower variable cost and higher net benefit. Treatment 2 had the lower total variable costs and higher net benefits than the treatment with the next lowest total variable costs, treatments 6. Treatment 3 had lower total variable cost and gave high net benefit compared to treatment 4, 5, 7, 8, 9 and 10. Based on the dominance analysis treatment 2 and 3 were potential options (table 2). Therefore, treatments 4, 5, 6, 7, 8, 9 and 10 were eliminated from further economic analysis and only the dominant treatments

were considered further in the partial budget analysis (table 3).

The partial budget analysis (table 3), showed that treatment with the higher net benefit was treatment 3 (175,123ETB/ha) with acceptable marginal rate of return compared to treatment 2 which gave 164,492 ETB/ha. However, the marginal rate of return for this treatment was 1512%. This means for each 1 ETB investment, the producer can get 15.12 ETB. Since the minimum acceptable rate of return assumed in this experiment was 100%, both these treatments can give an acceptable marginal rate of return for the extra investment. Therefore, treatment 2 and 3 can be accepted as the preferred option for farmers.

Table 2: Economic (partial budget and dominance) analysis of fertilizers on potato at Bule

| Treat | NPSB (kg/ha) | NPS (kg/ha) | Cu (kg/ha) | N kg/ha | K kg/ha | Potato seed kg/ha | Av. Yield | Adj. yield | TCTV (EB/ha) | Revenue (EB/ha) | NB (EB/ha) | MRR (%) |
|-------|--------------|-------------|------------|---------|---------|-------------------|-----------|------------|--------------|-----------------|------------|---------|
| 1 | 0 | 0 | 0 | 0 | 0 | 1900 | 19.9 | 17.9 | 19000 | 116368 | 97368 | |
| 2 | 150 | 0 | 0 | 91 | 90 | 1900 | 32.1 | 28.9 | 23439 | 187931 | 164492 | |
| 6 | 150 | 0 | 0.625 | 91 | 90 | 1900 | 30.9 | 27.8 | 23939 | 180666 | 156727 | D |
| 3 | 200 | 0 | 0 | 121 | 90 | 1900 | 34.1 | 30.7 | 24216 | 199339 | 175123 | |
| 7 | 200 | 0 | 0.625 | 121 | 90 | 1900 | 30.0 | 27.0 | 24716 | 175208 | 150492 | D |
| 4 | 250 | 0 | 0 | 152 | 90 | 1900 | 32.8 | 29.5 | 25001 | 191979 | 166978 | D |
| 10 | 0 | 242 | 0 | 143.5 | 90 | 1900 | 31.4 | 28.3 | 25005 | 183690 | 158685 | D |
| 8 | 250 | 0 | 0.625 | 152 | 90 | 1900 | 33.4 | 30.1 | 25501 | 195343 | 169842 | D |
| 5 | 300 | 0 | 0 | 182 | 90 | 1900 | 34.0 | 30.6 | 25778 | 198754 | 172976 | D |
| 9 | 300 | 0 | 0.625 | 182 | 90 | 1900 | 32.9 | 29.6 | 26278 | 192272 | 165994 | D |

Yield adjustment = 10%, field price of potato = 6.5 Ethiopian Birr (ETB)/kg, potato seed = 10 ETB/kg, official price for urea-N = 8.75 ETB/kg, NPS fertilizer = 10.9 ETB/kg, NPSB fertilizer = 10.3 ETB/kg, potassium chloride-K = 14ETB/kg, copper sulfate-Cu = 1000 ETB/kg, TCTV = total costs that varies, NB = net benefit, D indicates dominated treatments that are rejected, MRR = marginal rate of return.

Table 3: Economic (partial budget and marginal rate of return) analysis of fertilizers on potato at Bule

| Treatments | Av. Yield (t/ha) | Adj. yield (t/ha) | TCTV (EB/ha) | Revenue (EB/ha) | NB (EB/ha) | MRR (%) |
|---------------------------|------------------|-------------------|--------------|-----------------|------------|---------|
| 1. No fertilizer | 19.9 | 17.9 | 19000 | 116368 | 97368 | |
| 2. NPSB: 69,23.5,10, 1.07 | 32.1 | 28.9 | 23439 | 187931 | 164492 | 1512 |
| 3. NPSB: 92, 31, 13,1.4 | 34.1 | 30.7 | 24216 | 199339 | 175123 | 1369 |

Yield adjustment =10%, field price of potato = 6.5 Ethiopian Birr (ETB)/kg, potato seed = 10 ETB/ha, official price for urea-N = 8.75 ETB/kg, NPS fertilizer = 10.9 ETB/kg, NPSB fertilizer = 10. 3 ETB/kg, potassium chloride-K= 14 ETB/kg, copper sulfate-Cu= 1000 ETB/kg, TCTV = total costs that varies, NB = net benefit, MRR = marginal rate of return.

VII. SENSITIVITY ANALYSIS

In different reasons market prices are ever changing and recalculation of the partial budget considering future prices is necessary to pinpoint treatments which can be remain stable and sustain acceptable returns for farmers despite input price fluctuations. In the present study, assuming that the official price of NPSB, urea and potassium fertilizers will increase by 20%. The assumption of price increment in

these fertilizers is mainly the change in the exchange rate and price change in transport.

Based on the sensitivity analysis (table 4), treatments 2 (NPSB: 69 kg N + 23.5 kg P + 10 kg S + 1.07 kg B/ha) and 3 (NPSB: 92 kg N + 31 kg P + 13 kg S + 1.4 kg B/ha) gave an economic yield response and also sustain acceptable returns even under 20% input price increment likely farmers face in the future. Therefore, farmers could choose either of the two new fertilizer rates depending on their resource.

Table 4: Partial budget analysis at projected future prices of NPS, NPSB and urea fertilizers at Bule

| Treatments (kg/ha) | Av. Yield | Adj. yield | TCTV (EB/ha) | Revenue (EB/ha) | NB (EB/ha) | MRR (%) |
|---------------------------|-----------|------------|--------------|-----------------|------------|---------|
| 1. No fertilizer | 19.9 | 17.9 | 22800.0 | 116368.2 | 93568.2 | |
| 2. NPSB: 69,23.5,10, 1.07 | 32.1 | 28.9 | 28126.8 | 187931.3 | 159804.5 | 1243 |
| 3. NPSB: 92, 31, 13,1.4 | 34.1 | 30.7 | 29058.8 | 199338.8 | 170279.9 | 1124 |

Yield adjustment =10%, field price of potato = 6.5 Ethiopian Birr (ETB)/kg, potato seed = 10 ETB/ha, official price for urea-N = 8.75 ETB/kg, NPS fertilizer = 10.9 ETB/kg, NPSB fertilizer = 10. 3 ETB/kg, potassium chloride-K= 14 ETB/kg, copper sulfate-Cu= 1000 ETB/kg, TCTV = total costs that varies, NB = net benefit, MRR = marginal rate of return.

VIII. CONCLUSION AND RECOMMENDATION

This study showed that potato yield increased using the blended fertilizers compared to the control. However, there was no significant difference between the different types and levels of blended fertilizers.

The economic analysis revealed that except treatment 2 and 3 all the treatments were dominated by the treatment with low total cost that varies. The highest net benefit was obtained from treatment 3 with acceptable marginal rate of return. However, treatment 2 also met more than the required return. This result also confirmed by the sensitivity analysis, both treatments sustains acceptable returns even under 20% input price increment. Therefore, treatment 2 (NPSB: 69 kg N + 23.5 kg P + 10 kg S + 1.07 kg B/ha) and treatment 3 (NPSB: 92 kg N + 31 kg P + 13 kg S + 1.4 kg B/ha) with 50 kg K/ha are recommended and farmers could choose either of the two new fertilizer rates depending on their resource.

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Effects of Soaking Time and Temperature on the Nutritional Content and Sensory Quality of Soybean Flour and Milk

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Abstract- Soybean plays a great role to reducing protein-energy malnutrition. However, the major problems in raw soybeans are off-flavor. Processing also affect nutritional value unless optimum processing methods were used. Thus, this investigation was carried out to evaluate the effect of soaking temperature and time on proximate composition and sensory properties of soy products made from soybean variety AFGAT (*Glycine max*). Two soaking temperatures hot water (40°C) and ambient temperature (25°C) and three soaking times (8, 12 and 16 hrs) were considered in the experiment. The study revealed that soaking time and the temperature had significantly ($p < 0.05$) affected proximate composition of soybean products. Flour protein content was significantly affected by soaking time and temperature. A higher value (44.26%) was observed at 16 hrs soaking time with 40°C soaking temperature. The result was slightly greater than the raw soy flour (44%). Flour fat content was 21.98% for soybean soaked for 8 hrs at 40°C. This result was found to be higher than the raw (20.80%). Significantly higher flour fiber content (3.77%) was obtained at 16 hrs soaking time with 40°C. The fiber content appeared to increase with increasing soaking time at 40°C. Soy milk proximate composition was significantly affected by soaking time and the temperature.

Keywords: *soaking time and temperature, proximate composition, soybean, soybean flour, soy milk.*

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Abstract- Soybean plays a great role to reducing protein-energy malnutrition. However, the major problems in raw soybeans are off-flavor. Processing also affect nutritional value unless optimum processing methods were used. Thus, this investigation was carried out to evaluate the effect of soaking temperature and time on proximate composition and sensory properties of soy products made from soybean variety AFGAT (*Glycine max*). Two soaking temperatures hot water (40°C) and ambient temperature (25°C) and three soaking times (8, 12 and 16 hrs) were considered in the experiment. The study revealed that soaking time and the temperature had significantly ($p < 0.05$) affected proximate composition of soybean products. Flour protein content was significantly affected by soaking time and temperature. A higher value (44.26%) was observed at 16 hrs soaking time with 40°C soaking temperature. The result was slightly greater than the raw soy flour (44%). Flour fat content was 21.98% for soybean soaked for 8 hrs at 40°C. This result was found to be higher than the raw (20.80%). Significantly higher flour fiber content (3.77%) was obtained at 16 hrs soaking time with 40°C. The fiber content appeared to increase with increasing soaking time at 40°C. Soy milk proximate composition was significantly affected by soaking time and the temperature. Soy milk protein content was 52.35% for soybean soaked for 16 hrs at 25°C. Protein were increased as the soaking time increasing at a temperature 25°C. Soy milk fat content was 23.98% for soybean soaked for 16 hrs at 25°C. The processing methods showed that the most acceptable product was obtained for soybean soaked for 8 hrs at 40°C which resulted in good color, flavor, odor and overall acceptance of soy milk with an average sensory score of 7.00, 6.68, 6.96 and 7.00, respectively on 7- point hedonic scale. Whereas, soybean soaked for 16 hrs at 25°C were found to have less sensory acceptance. In conclusion, the proximate composition of soybean flour and milk and sensory quality of soybean milk were influenced by soaking time and temperature. The result suggests that good quality soybean flour and soybean milk were obtained from soybean soaked for 8 hrs at 40°C.

Keywords: soaking time and temperature, proximate composition, soybean, soybean flour, soy milk.

I. INTRODUCTION

The soybean (*Glycine max* (L) Merrill, family Leguminosae) originated in Eastern Asia, have been grown as a food crop for thousands of years in China. Nowadays, soybeans are widely grown and consumed as a source of plant protein in the diets of

millions of people throughout the world (Varma, and Mehta, 1988 and Millward, 2004). In the year 2004/05, the world had produced approximately 229 million metric tons of soybean, enough to give each man, woman, and child 35 kg of soybeans for each, or the equivalent of nearly 300 liters of soybean milk for a year (Riaz, 2006). The United States, the overall soybean foods industry has grown dramatically since the mid-1990s, from \$1.2 billion in 1996 to an estimated \$4.0 billion in 2004. 2001/02 Ethiopia had produced only 42,881.47 quintals of soybeans from 4,922.38 hectares of land (Riaz, 2006).

Soybean is important sources of high quality, inexpensive protein than other legumes and about 35 - 38 % of its calories are derived from its protein as compared to 20 -30% in most other beans. Protein rich source of other legume is high in cholesterol and saturated fat whereas, soybean foods provide high quality protein, cholesterol free and low in saturated fat, equivalent to animal protein in its protein quality and higher than other plant proteins (FAO, 1990). Among soybean products, soy flour is made from whole or dehulled soybeans. Soy flour had protein content between 35 and 40% and a fat level between 15 and 20%. It is extremely nutritious and high in fiber and contains all of the vitamins, minerals, and phytochemicals of soybeans. Soy milk is an emulsive liquid extracted from soybean and it is recognized as milk from vegetable due to its high protein and fat content and the homogeneous form in texture resembling animal milk. The protein and fat content ranges from 39 to 46% and 16 to 18%, respectively, based on variety.

Soy milk is cholesterol free and low fat produced from soaked soybean. The nutritional value can be compared with that of cow's milk. It contains higher protein than cow's milk (Hurrell *et al.*, 1992). Soy milk containing good quality protein, fat, isoflavones, vitamins, minerals and carbohydrate has attracted a great deal of public attention as a healthy food and a good choice for people who are lactose intolerant, in which 75% of the world population have no ability to digest lactose that causes stomach cramp, flatulence and diarrhea (Riaz, 2006). Also it is a good alternative to those who are allergic to the proteins of cow's milk, however, there are some problems in using soy milk

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because it has unpalatable taste such as greasy smelling and disagreeable stimulating taste, odor and flavor due to minor components of soybean (Muller Harvey and Allan 1992). This probably because of lipoxygenase enzyme found on the intact seed that catalyzes lipid oxidation leading to the production of unacceptable flavor. To control this reaction, soybeans are blanched before soaking (Hurrell *et al.*, 1992). Therefore, search for suitable soy milk and soy flour processing methods that can be locally adapted, improve the nutritional quality through suppressing the lipo-oxiginase enzyme and off flavor nature coupled with better nutrient extraction are required for proper utilization of soybean flour and milk in Ethiopia.

General Objective

To investigate the effects of soaking time and temperature on soybean flour and soybean milk quality

Specific Objectives

1. To study the effects of soaking time and temperature on the proximate nutritional value of soybean flour and soybean milk
2. To study the effects of soaking time and temperature on sensory quality of soybean milk

II. MATERIALS AND METHODS

This experiment was conducted at Haramaya university in 2010. Improved soybean variety named AFGAT [TGX-1892-10F], was used. This variety was selected due to its relatively lower price, good productivity and high nutritional composition. For ensuring the quality, the breeder seed was used. The experiment comprised two factors (soaking times and temperatures) arranged in CRD with three replications. Soaking time had three levels (8, 12 and 16 hrs) and two soaking temperatures, 25 and 40°C.

The clean seed samples was immersed into a thermostatically controlled hot water bath for 10 seconds at a temperature 91°C with a ratio of soybean grain to water, 1:3 to suppress the soybean lipoxygenase enzyme activity (Brown *et al.*, 1982). As soon as the blanching was completed, the samples were withdrawn and immediately cooled down in cold water at ambient temperature for 15 seconds. The blanched samples were soaked in tap water for 8 hrs, 12 hrs and 16 hrs at ambient temperature (25°C) and hot water (40°C) using a thermostatically controlled water bath. The soaked samples were dried in direct sunlight. The seed coats and germs were then removed manually using stone grinder. The dehulled beans were milled for further analyses.

The untreated sample (control) which was used for comparison was cleaned manually to remove physical impurities such as foreign matters, immature and damaged seeds. The cleaned soybean was milled using Professional Burr mill and the flour was sifted to pass through 710 micron test sieve. The sample was

sealed in moisture proof a plastic bag and stored at room temperature until laboratory analysis were conducted (Nwabueze, 2007). The grain flour was analyzed for Proximate Composition to compare with the treated samples.

Treated soy flour preparation: Soybean was cleaned from physical impurities and thoroughly washed with soft water, then it was blanched. One kg blanched soybean was soaked in 2 liters hot water (40°C) and 25°C in each a plastic container for 8, 12, and 16 hours. After pouring off the water, the soybean was rinsed with water (Hymowitz and Polmer 2004). Part of each soaked soybean were dried in direct sun light and dehulled manually using stone grinder. The dehulled beans were manually separated from the hull and milled to flour using laboratory mill.

Soy milk preparation: Soy milk was prepared from soaked soybean and Japanese method was used for main soy milk maker (Soy wonder Soymilk Machine, Model MJ 720) for each grinding time (10 min) to produce a slurry. The slurry was heated near boiling temperature and the soluble soy milk emulsion was readily separated from the insoluble residue (okara) by passing a slurry through a fine filter cloth (cheese cloth). The filter cloth was folded four times to reduce entrance of unwanted materials to the extracted soy milk (Nwabueze, 2007). The soy milk was boiled at 90°C for 18 minutes to reduce trypsin inhibitor and to retain nutritive value of the product (Camire, 2001). Soy milk was analyzed for its nutrient composition.

The soymilk sample were dried in two stage drying in an oven (Incubators Circulation air natural, Binder GMBH) at a temperature of 70°C and 100°C for 16 hrs and 1 hrs, respectively.

The soybean milk powders were sealed in moisture-proof plastic bag and analyzed for nutritional composition.

a) Raw Material Proximate Composition

i. Determination of moisture content

The moisture content was determined by the gravimetric method as described in the AOAC (1990), Method No 935.30. Weigh a clean dried and covered flat aluminum dish, and flour about 2g was dried in an air forced drought oven at 103°C for 6 hrs by placing the cover on the sample dish half-open. Then it was cooled in a desiccator (Nalgene Model 5317-0120) and re-weighed. The mass loss on drying, determined as % moisture was used:

$$\text{Moisture}(\%) = \frac{m_{\text{initial}} - m_{\text{dried}}}{m_{\text{initial}}} \times 100$$

Where m_{initial} = weight of the sample before drying

m_{dried} = weight of the sample after drying

ii. *Determination of crude protein*

Total nitrogen content was determined by micro-Kjeldahl method, according to AOAC (1990)

$$\text{Nitrogen}(\%) = \frac{VHCl \text{ in } L \times NHCl(\text{ca.}0.1) \times 14.00}{\text{Sample weight on dry materbasis}} \times 100$$

Where:

V is a volume of HCL in L consumed to the end point of the titration, N is the normality of HCl (used often is 0.1 N), and 14.00 is the molecular weight of nitrogen. The percent of nitrogen was converted to % of protein by using conversion factor (% protein = 6.25 × % N for soy flour).

iii. *Determination of crude fat*

The fat content of flour was determined, according to AOAC (1995) Method No 923-09. Flour (2g) was extracted with 150 ml di-ethyl ether for a minimum period of 8 hrs in the soxhlet extractor. The solvent was then evaporated by heating on a steam bath. The flask containing the extracted fat was dried in an oven to a constant mass for 1 hr.

$$\text{Crude fat, percent by weight} = \frac{W_2 - W_1}{W} \times 100$$

Where: W_1 = Weight of the extraction flask (g)

W_2 = Weight of the extraction flask plus the dried crude fat (g)

W = Weight of sample flour (g)

iv. *Determination of ash content*

Ash content of flour was determined according to AOAC (1995) Method No 923-09. Clean porcelain dish, dried at 120°C in an oven was ignited at about 550°C in a muffle furnace for 3 hours was cooled in a desiccator and weighed (m_1). Then 3 g of flour sample was put into the porcelain dish and weighed (m_2). This sample was dried at 120°C for 1 hour and carbonized over to hot plate until contents turn black. The dish with its contents was transferred to a muffle furnace and ignited at about 550°C until ashing was complete. The residue was weighed (m_3). The total ash was expressed as percentages on a dry matter basis as follows:

$$\text{Total Ash}(\%) = \left(\frac{M_3 - M_1}{M_2 - M_1} \right) \times 100$$

Where: ($M_2 - M_1$) is sample mass in g on dry base and ($M_3 - M_1$) mass of ash in g.

v. *Determination of crude fiber*

A 3 g sample was transferred to 600 ml beaker. After digestion with 1.25% sulfuric acid, washed with distilled water and digested by sodium hydroxide (1.25%), it was then filtered in 76 μm coarse porosity crucible in apparatus at a vacuum of about 25 mm. The residue left after refluxing was again washed with 1.25% sulfuric acid near boiling point. This residue was then

method (No. 925-09) using Automatic digestion and distillation systems (Model UDK-142, Europe). For a control urea was used.

dried at 110°C overnight, cooled in desiccator and weighed (M_1). After ashing at 550°C, it was cooled in a desiccator and weighed again to get mass of ash (M_2). The total crude fiber was expressed in percentage as:

$$\text{Total Crude fiber} = \left(\frac{M_1 - M_2}{M_3} \right) \times 100$$

Where; M_1 = mass of ash + fiber,

M_2 = mass of ash

M_3 = mass of sample on the dry matter basis

v. *Determination of carbohydrate*

Carbohydrate content was determined by the difference.

$$\% C = 100 - [\% M + \% P + \% F + \% Fb + \% A]$$

Where: C-Carbohydrate content, M-Moisture content, P-Protein content, F-Fat content, Fb- Fiber content and A-Ash content.

vi. *pH Value Determination*

For soy milk pH determination, pH meter was used (model HI 9017 microprocessor pH meter, USA). Glass electrode attached to digital electronic pH meter was used, after calibrating using standard buffer solutions of pH 4 and pH 7. pH was measured by inserting the glass electrode into the sample in a beaker and reading was taken when the displayed value is steady at room temperature.

vii. *Sensory Evaluation*

Sensory panelists were selected from Awassa Agricultural Research center food science staffs that have already been trained in sensory analysis. A total of 50 male and female panelists were involved in the sensory evaluation to assess the sensory quality of soymilk (Dadzie, 1998). The evaluation were includes sensory attributes such as color, flavor, odor and over all acceptability of the product, using 7-point hedonic scale rating consisting of 1 (dislike very much), 2 (dislike moderately), 3 (dislike slightly), 4 (neither like nor dislike), 5 (like slightly), 6 (like moderately) and 7 (Like very much) was used.

After cooling for 5 min, the soy milk was kept in a refrigerator for short time until it was tasteful for subjective analysis. Product sample was arranged and each panelist was instructed on the procedure of sensory evaluation. Soymilk was given in a glass for each evaluator and the number of samples was 6 at a time. Panelists were instructed to make their own

individual assessments according to their best feel after tasting the product. Total samples were presented for each panelist throughout the evaluation period to evaluate the sample on the bases of taste, color and flavor and over all acceptability.

b) Statistical Analysis

All data were analyzed using two factors analysis of variance (ANOVA). Duncan's multiple-range test was used to establish multiple comparisons of mean values. Mean values were considered at 5% significance level ($p < 0.05$). The statistical analysis of the data were conducted using the SAS statistical software package.

III. RESULTS AND DISCUSSIONS

a) Proximate Composition of Raw Soybean Flour

i. Proximate composition

The proximate compositions analyzed for raw soybean flour (control) were given in Table 3. The flour moisture was 5.33%. This result was similar to Olasoju and Ajav (2007) result (5.38%). But lower than 7.75% reported by Yimer and Admassu, (2008) and 7.4 % reported by Abdet *et al.*, (2009). This difference was probably due to the variety and environmental factors (Famurewa and Raji, 2005). Lower moisture content hinders microbial growth and enzymatic activity; and reduces rancidity during storage. This may contribute to keeping the product in good quality and better shelf life.

The crude protein content of raw soy flour was 44.00%. The result obtained was in close agreement with those of an earlier report (43.4%) by Fasoyiro *et al.*

(2006). Also the present value was in the range of 40% to 45% reported by Liener, (1989). The crude fat content of raw soy flour obtained in the present study was 20.80%. This value was in the range (15 to 20%) reported by Famurewa and Raji, (2005). But it was higher than 18.5% (Abdet *et al.*, 2009). The ash content of raw soy flour was 4.98% (Table 3). This result was similar with that reported (4.86%) by Fasoyiro *et al.*, (2006). However, these values were higher compared to processed (treated) flour. This could probably be due to the presence of hull in raw soybean and were lower than 5.6%, reported by Yanez, *et.al* (1982) and 6%, Stuffer (2008), 5.73% and 5.88% reported for Awassa and Belesa varieties, respectively (Yimer and Admassu, 2008). But the present value was in the range of 3.09% to 5.04% obtained by Vasconcelos *et al.* (1997).

The crude fiber was 5.83% (Table3). The result obtained was in agreement with Fasoyiro *et al.* (2006). Who reported that, the fiber content of soy flour was 5.05%. This value was lower than (6.89% and 7.27%) the two varieties, namely, Awassa and Belesa (Yimer and Admassu, 2008). Similarly, higher fiber content was measured from raw soy flour due to the presence of hull compared to processed (blanched, soaked, and dehulled). The total carbohydrate of soy flour was 20.00%. The result obtained was less than the value reported by Famurewa, Raji (2005) (24.92%) and Stauffer (2008) (22%). This might be due to varietal differences. Variety, environmental factors and management in pre and post-harvest conditions could affect the proximate composition (Fasoyiro *et al.*, 2006).

Table 3: Proximate composition of raw soybean flour

| Moisture (%) | Crude protein (%) | Crude fat (%) | Crude fiber (%) | Ash (%) | Carbohydrate (%) |
|--------------|-------------------|---------------|-----------------|-----------|------------------|
| 5.33±0.71 | 44±0.24 | 20.80±1.01 | 5.83±0.92 | 4.98±0.52 | 20.0±1.23 |

b) The Effect of Soaking Temperature and Time on Proximate Composition of Soybean Flour

Moisture content: The proximate compositions of soy flour were presented in Table 4a. Effect of soaking temperature was significant ($p < 0.05$) on soy flour moisture content. The moisture content was 4.73% at 25°C and 4.55% for those soaked at 40°C. This result is somewhat higher than the findings of Orhevba, (2010) who reported that moisture content of soy flour was lower (4.6%) at a higher temperature. However, the result showed lower value as compared to 6.6% reported by Abdet *et al.*, (2009). The moisture content decreased with increasing temperature. This was perhaps due to the evaporation of water. This could be explained by the fact that the size of the soybean increase due to expansion of tissue which led to the loss of moisture as soaking temperature increased. Lower moisture content helps the product to retain its quality

without deterioration for a long period because the mold and microbial growth can be potentially inhibited.

Soybean soaking time did not significantly affect the moisture content of the flour. This might be due to uniform sun drying after soaking. Interaction effects of soaking time and temperature was significantly ($p < 0.05$) affected the soy flour moisture content (Table 4a). Accordingly, flour moisture content was significantly lower (4.22%) than other treatments for soybean soaked for 12 hrs at 40°C. This value was also lower than the raw (5.33%). The results in this study were in agreement with earlier investigator Orhevba (2010), who reported that moisture content of soy flour as (4.8%).

Crude protein: Soaking temperature exhibited no significant effect ($p > 0.05$) on the protein content of the flour as both flours produced after soaking at 25°C and 40°C had 43.4% and 43.7% protein content, respectively

(Table 4a). This finding was in agreement with those of Yanez, et al. (1982) who reported no significant effect of soaking temperature at 45°C the protein content of peanut flour.

The effect of soaking time on the protein content of soy flour was significant ($p < 0.05$). The flour protein content was 43.17% for soybean soaked for 8 hrs and 43.96% for soybean soaked for 16 hrs (Table 4a). No difference was observed as a result of the 12 hrs soaking time as compared to either value. But the trend was increasing, as the soaking time increased from 8 to 16 hrs. This showed that soaking soybean for a longer time gave more protein content compared to soaking for a short time. Although the increase in protein was more significant in 16 hrs soaked soybean than 12 hrs, the value remained higher as compared to 8 hrs. Alonso *et al.*, (1998) observed a significant increase in protein levels in kidney beans after soaking (3%) and after dehulling (6%). Mubarak (2005) also reported a 9% increase in protein content in mungbean flour, but Njitang *et al.*, (2001) observed an average decrease in protein content in dehulled bean flour due to germination. The result in the present investigation was in agreement with the earlier investigation of Orhevba, (2010) who studied processing of soybean and found out that the protein content of soaked soybean ranged from 43 to 46%.

Crude protein content of soy flour was not significantly ($p > 0.05$) influenced by the interaction effects of time and temperature. At soaking time of 16 hrs at 40°C, higher protein content (44.26%) was obtained compared to 8 hr soaking at 25°C, 8 hr soaking at 40°C and 12 hr soaking at 25°C (Table 4a). Furthermore the values exhibited a consistent increasing trend in protein content as the soaking time increased for both temperatures. At soaking temperature of 25°C and 8, 12, and 16 hrs of soaking time, the crude protein was 43.21, 43.42, and 43.65% respectively. At soaking temperature of 40°C, and 8, 12, and 16 hrs of soaking time, 43.13, 43.72, and 44.26%, respectively protein content of soy flour were observed. This value is slightly higher than the raw soy flour due to both soaking and dehulling. According to Mubarak (2005) after soaking, dehulling with the combination of germination, the protein value was higher compared to the raw flour.

Fat content: The fat contents of soy flour showed significant difference ($p < 0.05$) with the soaking temperature. The soybean subjected to 25°C soaking temperature had lower (19.86%) fat content than obtained from soybean soaked at 40°C (21.22%) (Table 4a). The value exhibited increasing trend as the soaking temperature increased. High value in which the soybean seeds are soaked in hot water because the preheating treatment reduces viscosity of the oil and allows for easy breakdown of cells and release of oil. This can be explained by the fact that due to expansion

of the tissue of soaked soybean facilitate better extraction of fat. This value obtained were closer to the value (26% to 19.90%) reported by Raji and Famurewa, (2008).

The fat content of the flour appeared to be decreased with increase in the soaking time. The shortest soaking time, i.e. 8 hrs, gave flour with the highest (21.47%) fat content which was significantly different ($p < 0.05$) from those of 20.21 and 19.95% for the flour obtained after 12 and 16 hrs of soaking time, respectively. This could be due to leaking of fat as soaking time increased. This result was in agreement with the findings of (Suberbie *et al.*, 1981) who reported that dehulled beans contained 18% to 22% oil. This value was also higher than the raw soy flour (20.80%). Suberbie *et al.*, (1981) also reported that nutrient distribution after dehulling and soaking may explain higher in fat content of bean compared to raw seed flour.

Soaking temperature and time interaction exhibited no significant effect ($p > 0.05$) on the fat content of the flour as flours produced after soaking at 12 hrs with 25°C, 16 hrs with 25°C, and 40°C (Table 4a). The fat content 21.98% was obtained from soybean soaked for 8 hrs at 40°C but not significantly different from 20.96% and 21.38% soybean soaked at 8 hrs at 25°C and 12 hrs at 40°C, respectively.

Crude fiber: The crude fiber content of the soy flour soaked at 40°C had showed significantly higher (2.66%) value than the flour produced after soaking the soybean at 25°C. The value increased with increasing in soaking temperature attaining the highest value at the highest soaking temperature. This could be explained by the fact that the size of the soaked soybean increased with temperature due to expansion of the tissue particles allowed to give higher fiber at higher temperature. The result obtained was in close agreement with those of earlier reported (2.3%) by Yanez, *et al.* (1982). Results in this study also agreed with the findings of (Dubois and Hoover, 1981) of 2.1%. The result obtained in the present study was lower than the raw soy flour (5.83%). This might be due to the lower or absence of hull compared to raw soy flour.

The fiber contents of the flours obtained from the soybean subjected to the three soaking periods exhibited significant ($p < 0.05$) difference from each other (Table 4a). The flour of the soybean with the longest (16 hrs) soaking time had the highest (3.22%) fiber content while that of the shortest (8hrs) soaking time showed the lowest (1.47%). The 12 hrs soaking time resulted in flour with 2.08%, which was significantly different ($p < 0.05$) from either of the two (Table 4a). As the soybean soaking time increased from 8 to 16 hrs the score of crude fiber content of the soybean flour also increased from (1.47%, 2.08%, and 3.22%), respectively. This

could be due to longer soaking time that facilitated to separate fiber from the cell during processing.

Interaction effect of soaking time and temperature significantly affected crude fiber content ($p < 0.05$) (Table 4a). Accordingly, higher value (3.77%) was obtained for soybean soaked at 16 hrs and 40°C. The lower value was observed for soybean soaked for

12 hrs at 25°C and 8 hrs soaked at 40°C (Table 5a). These values were lower than the raw (5.83%) soy flour fiber due to soaking and dehulling. The values showed a consistent increase in fiber content as the soaking time increased at 40°C (1.27, 2.95, and 3.77%). This might be due to higher temperature combined with longer soaking time which facilitated better separation of fiber.

Table 4a: Effect of soaking temperature and time on proximate composition (moisture, protein, fat and crude fiber) of soybean flour and soybean milk

| Variables | Soy flour | | | | Soy milk | | | |
|---|--|--------------|---------------|-----------------|--------------|-------------|-------------|-----------------|
| | Moisture (%) | Protein (%) | Fat (%) | Crude fiber (%) | Moisture (%) | Protein (%) | Fat (%) | Crude fiber (%) |
| Time | Main effect of soaking temperature (25 and 40°C) and soaking time (8, 12 and 16 hrs) | | | | | | | |
| Temp. | Main effect of soaking temperature (25 and 40°C) and soaking time (8, 12 and 16 hrs) | | | | | | | |
| 25 | 4.73±0.17a | 43.43±0.33a | 19.86±1.09b | 1.85±0.65b | 10.54±1.40b | 50.76±.82a | 21.38±1.97a | 0.05±0.01a |
| 40 | 4.55±0.30b | 43.70±0.57a | 21.22±1.03a | 2.66±1.11a | 11.20±1.26a | 49.16±1.31b | 21.41±1.18a | 0.04±0.03a |
| 8 | 4.63±0.15a | 43.17±0.25b | 21.47±0.66a | 1.47±0.22c | 11.81±0.75a | 48.91±0.66c | 20.37±0.29c | 0.03±0.01b |
| 12 | 4.56±0.39a | 43.57±0.37ab | 20.21±1.53b | 2.08±0.95b | 10.69±1.21b | 50.98±0.66a | 21.45±1.61b | 0.04±0.02b |
| 16 | 4.73±0.15a | 43.96±0.44a | 19.95±0.93b | 3.22±0.62a | 10.10±1.49c | 49.98±2.62b | 22.35±1.85a | 0.06±0.01a |
| Interaction effect (soaking time and temperature) | | | | | | | | |
| 8 | 4.58±0.09b | 43.21±0.17b | 20.96±0.13abc | 1.67±0.02d | 11.15±0.19b | 48.42±0.41e | 20.14±0.08c | 0.04±0.01bc |
| 8 | 4.66±0.21ab | 43.13±0.36b | 21.98±0.56a | 1.27±0.04e | 12.47±0.24a | 49.41±0.43d | 20.61±0.20c | 0.03±0.01cd |
| 12 | 4.90±0.14a | 43.42±0.26b | 19.03±0.32d | 1.22±0.19e | 11.72±0.55b | 51.49±0.27b | 20.01±0.31c | 0.05±0.01b |
| 12 | 4.22±0.10c | 43.72±0.40ab | 21.38±1.27ab | 2.95±0.11b | 9.66±0.38c | 50.46±0.47c | 22.88±0.47b | 0.03±0.01cd |
| 16 | 4.68±0.07ab | 43.65±0.44ab | 19.61±1.31cd | 2.67±0.15d | 8.75±0.21d | 52.35±0.30a | 23.98±0.51a | 0.05±0.01b |
| 16 | 4.78±0.21ab | 44.26±0.17a | 20.29±0.31cbd | 3.77±0.19a | 11.45±0.23b | 47.61±0.46f | 20.73±0.65c | 0.08±0.01a |
| Mean | 4.64 | 43.56 | 20.54 | 2.26 | 10.86 | 49.96 | 21.39 | 0.04 |
| CV | 3.25 | 0.76 | 3.91 | 6.06 | 3.00 | 0.80 | 1.97 | 16.84 |

CV=coefficient of variance, Values followed by different letters indicate a significant difference ($p < 0.05$), Mean \pm SD; Number of observation with in treatment = 3

Table 4b: Effect of soaking temperature and time on ash, carbohydrate and pH of soybean flour and soybean milk

| Variables | Soy flour | | Soy milk | | | |
|---|-----------|--|--------------|------------------|-------------|-------------|
| | Ash (%) | Carbohydrate (%) | Ash (%) | Carbohydrate (%) | pH | |
| Time(hrs) | Temp. | Main effect of soaking temperature (25 and 40°C) and soaking time (8, 12 and 16 hrs) | | | | |
| | 25 | 4.41±0.18a | 25.30±2.19a | 6.62±0.54b | 10.65±2.47a | 6.66±0.06a |
| | 40 | 4.54±0.21a | 23.38±1.49b | 8.30±1.44a | 9.87±0.45b | 6.68±0.04a |
| 8 | | 4.43±0.22a | 24.72±0.66ab | 6.89±0.53b | 11.96±2.12a | 6.71±0.03a |
| 12 | | 4.52±0.15a | 25.33±2.42a | 7.36±0.72b | 9.47±0.27b | 6.67±0.05ab |
| 16 | | 4.46±0.25a | 23.00±2.15b | 8.13±2.15a | 9.36±0.94b | 6.64±0.05b |
| Interaction effect (soaking time and temperature) | | | | | | |
| 8 | 25 | 4.44±0.26a | 25.12±0.07ab | 6.43±0.20cd | 13.81±0.88a | 6.72±0.04a |
| 8 | 40 | 4.42±0.22a | 24.32±0.77ab | 7.36±0.15b | 10.11±0.44b | 6.69±0.02ab |
| 12 | 25 | 4.44±0.19a | 27.12±0.43a | 7.25±0.37bc | 9.45±0.33bc | 6.66±0.07ab |
| 12 | 40 | 4.59±0.05a | 23.53±2.21b | 7.47±1.06b | 9.49±0.26bc | 6.68±0.03ab |
| 16 | 25 | 4.34±0.08a | 23.65±3.15b | 6.17±0.18d | 8.69±0.82c | 6.62±0.04b |
| 16 | 40 | 4.59±0.32a | 22.29±0.55b | 10.08±0.17a | 10.03±0.47b | 6.66±0.06ab |
| Mean | | 4.47 | 24.34 | 7.46 | 10.26 | 6.67 |
| CV | | 4.75 | 6.69 | 6.47 | 5.68 | 0.71 |

Note: CV=coefficient of variance, Values followed by different letters indicate a significant difference ($p < 0.05$), Mean \pm SD; Number of observation with in treatment = 3

Ash: The effect of soaking temperature was not significant on the ash content of the two flours (Table 4b) (4.41% ash for 25°C soaking and 4.54% for 40°C soaking). These findings were in agreed with the previous studies Salim *et al.*, (2007) who reported ash content from 4.57 to 5%. Soaking time exhibited no significant effect on the ash content of soy flour processed at 8, 12, and 16 hours had 4.43, 4.52, and 4.46%, respectively. These results were not agreed with those of Orhevba, (2010) who reported that soaking time significantly increased from 4.17, 5.83, and 6.0% for ash content of soy flour but some difference was observed because he was used 2, 3, and 4 hrs soaking time, respectively.

The interaction effects of soaking time and temperature were not significantly affected ash content of soy flour (Table 4a). Ash content indicates milling performance by indirectly revealing the amount of bran contamination in flour. Ash in flour can affect color, imparting a darker color to finished products. The ash content was also an indicator of flour yield; hence the flour possessing lower ash content may have more endosperm and ultimately good flour yield (William *et al.*, 1986).

Total carbohydrate: Soy flour produced from soybean at 25°C had significantly ($p < 0.05$) higher total carbohydrate (25.30%) than hot water at a temperature of 40°C (23.38%) (Table 4b). This indicated that

temperature affected the total carbohydrate content of processed soy flour. As soaking temperature increased from 25 to 40°C, the carbohydrate content was decreased. This was probably due to leaching of water soluble nutrients to the soaking water. The total carbohydrate content obtained was in agreement with Stauffer (2008). But it was lower than the value (30%) reported by Famurewa and Raji, (2005). The total carbohydrate content of soy flour was not significantly affected ($p < 0.05$) by the soaking time. The total carbohydrate of 24.72%, 25.33%, and 23.00% were observed at soybean subjected to 8 hrs, 12, hrs and 16 hrs soaking time, respectively. However, these results were greater than the raw soy flour (20.0%).

The interaction effects of time and temperature were not significant on the total carbohydrate content of soy flour (Table 4b). At 12 hr soaking with 25°C, higher total carbohydrate content (27.12%) was obtained compared to 12 hrs soaking at 40°C, 16 hrs soaking at 25°C, and 16 hrs soaking at 40°C. However, the result was not significantly different ($p > 0.05$) compared to 8 hrs soaking at 25°C, and 8 hrs soaking at 40°C. The result of the present investigation was agreed with the earlier investigation of Salim *et al.*, (2007) reported that the total carbohydrate content of processed soy flour ranges between 18 to 29.2%. Salim *et al.*, (2007) also reported, higher total carbohydrate content in the processed soy flour than raw soy flour.

c) *The Effect of Soaking Temperature and Time on Proximate Composition of Soybean Milk*

Moisture content: Temperature was significant ($p < 0.05$) on moisture content of soy milk (Table 4a). The moisture content of soy milk was 11.20% for sample soaked at 40°C and 10.54% for sample soaked at 25°C. Increase in moisture content might be due to hot water which affected the water absorption capacity of protein and starch. This is supported by the finding of (Akinyele 1989; Saikia *et al.*, 1998) who reported that heat increased the water absorption of protein. In the present study, increased in moisture content probably had a dilution effect on other nutrient in the soaked soybean. The result obtained for soy milk moisture was in close agreement with those reported earlier (11.4% and 10.40%) by Salunkhe and Kadam, (1989).

The three different soaking times produced a significant difference ($p < 0.05$) in the moisture content of the soy milk (Table 4a). The increase in soaking time from 8 hrs to 16 hrs resulted in decreased moisture content. There was an inverse relationship between moisture and soaking time. Moisture content was significantly higher value occurred at 8 hrs soaking time and the lower moisture content obtained at 16 hrs soaking time. It showed 11.81% for 8 hrs soaking, 10.69% for 12 hrs soaking and 10.10% for 16 hrs soaking.

The combined effect of soaking time and temperature on the moisture content of soy milk was presented in Table 4a. The interaction effects of soaking time and temperature was significant ($p < 0.05$) on moisture content of soy milk. Moisture was significantly higher (12.47%) for soaking time 8 hrs at 40°C whereas the lower moisture value (8.75%) was for soaking time of 16 hrs at 25°C. These result agreed with the findings of Orhevba, (2010) who reported the moisture content of 9.2% but some difference was observed because of higher soaking time and temperature combination i.e. 18 hrs with 50°C.

Crude protein: The protein content of soy milk obtained from soybean soaked at 25°C was significantly ($p < 0.05$) higher (50.76%) than that of the soy milk produced from soybean soaked at 40°C (49.16%) (Table 4a). The high protein value for 25°C is probably due to the fact that soaking had increased the breaking down of the secondary bonds holding down the molecules of the amino acid thus making the protein more soluble and hence increasing availability of the amino acid. NRC (1993) showed that excessive heat treatment reduces the availability of heat sensitive amino acids and in particular that of lysine. Salunkhe and Kadam, (1989) indicated that heat treatment followed by grinding could affect nutritional composition. Wilkens and Hackler, (1969) stated that the effect of water temperature on soy milk composition were an increase in lipid but a decrease in carbohydrate and protein was relatively

unaffected but some difference observed because of higher temperature i.e. 25°C to 60°C.

Table 4a shows that soaking time had influenced soy milk protein content. The highest (50.98%) protein content was that of milk produced after 12 hrs of soybean soaking. The 16 hrs soaking period resulted in milk of 49.98% protein content whereas the 8 hrs soaking time gave 48.91% milk protein (Table 4a). All three values of protein contents were statistically different ($p < 0.05$) from each other eventhough no trend was shown relative to the soaking times. These value were different when compared to the protein range of soybean (35 to 40 %) reported by Famurewa and Raji, (2005) and most legumes (17% to 48.30%) reported by Reddy *et al.* (1984) and 39 to 46% observed by Bhumiratana (1978). The result reported in this study was probably due to high protein composition of AFGAT variety. Trongpanich, *et.al* (1988) reported that high protein in soybean usually gives high protein in soy milk.

Soy milk crude protein was significantly ($p < 0.05$) influenced by interaction effects of soybean soaking time and temperature. For 16 hrs soaking at 25°C, highest (52.35%) crude protein was obtained. The least result was for 16 hrs soaking at 40°C (Table 4a). Furthermore, the value exhibited increasing trend in protein content as the soaking time increased from 8 to 16 hrs at 25°C (48.42, 51.49 and 52.35%). The high protein value for 25°C was probably due to the fact that longer soaking time had increased the breaking down of the secondary bonds holding down the molecules of the amino acid thus making the protein more soluble and hence increasing availability of the amino acids. Soaking probably helped to increase the solubility of the denatured protein such that the protein content of the soymilk produced was high. Tunde and Souley (2009) reported that soaking as well as blanching gave a higher protein content of soymilk because soaking gives a tender product, which results in a finer slurry and thus more filtrate which will filter through the filter cloth thereby increasing yield and subsequently the protein content of soymilk. Wilkens and Hackler, (1969) reported that the effect of soaking temperature (25 to 60°C) on soy milk protein were relatively unaffected but protein recovery was known to decrease slightly at 70°C and higher.

Fat content: The fat content of soy milk obtained after treating the soybean with the two soaking temperature (25 °C and 40 °C) exhibited no significant difference (21.38 and 21.41% respectively). The fat content of the milk had significantly ($p < 0.05$) increased with increasing in soaking time. The 8, 12 and 16 hrs soaking periods resulted in 20.37%, 21.45% and 22.35%, respectively, fat content of the soybean milk (Table 4a). This could be due to longer soaking time weaken the structure of the tissues or wall of the cells that facilitated their rupture and thereby the released of the fat globules during the

milk processing. The present study was in agreement with Wilkens and Hackler, (1969) who reported that the major effect of soaking increased fat content with increasing soaking time.

The interaction effect of soaking time and temperature was significant ($p < 0.05$) on the fat content of soy milk. The highest fat value (23.98%) occurred in soybean milk prepared at 16 hrs soaking at 25°C (Table 4a). No significant differences were observed among 8 hrs soaking at 25°C and 40°C, 12 hrs soaking at 25°C and 16 hrs soaking at 40°C. These results were in line with Milligan *et al.*, (1981) who reported that 22.89%. Different investigators reported that, the overall effect of soaking was enrichment of the crude protein and fat content of the soy milk. Soaking at higher temperature was known to drastically reduce the yield of soy milk.

Crude fiber: The soaking temperature showed no significance difference on the crude fiber content of soymilks which were obtained from 25°C and 40°C soaking temperature (0.05 and 0.04%, respectively) (Table 4a). These value was very low as compared to Bahareh Hajirostamloo, (2009) who reported (0.2% to 0.31%) for soy milk. This could be probably due to processing difference or different soy milk extracting equipment (blender). Balogun and Fetuga, (1986) revealed that, the low crude fiber is nutritionally appreciated because it traps less proteins and carbohydrates and improves their bioavailability.

The longest soybean soaking time (16 hrs) had resulted in soybean milk with significantly ($p < 0.05$) higher crude fiber (0.06%) content than the other two soaking periods. The two soaking periods (12 and 8 hrs) values (0.04% and 0.03%) were not significantly different ($p < 0.05$). When the soaking time increased, the fiber content of the soy milk also increased. This could be due to longer soaking time which allowed separating fiber from the tissue.

The interaction effects of time and temperature had significantly ($p < 0.05$) affected the fiber content of soy milk (Table 4a). The highest value (0.08%) was observed at soaking time of 16 hrs at 40°C. The values in the present study were lower than the result of Bahareh Hajirostamloo, (2009) who observed the fiber content of soy milk in which ranged from 0.2 to 0.31%. In the current study, the crude fiber was closer to (0.0). This might be due to method of extraction and soy milk extracting equipment (blender). The blender sieve size was denser ($< 100 \mu\text{m}$) and with lower amount of pore space it accommodated suspended particles inside the extracting material. The denser and the less pore size distribution of the blander that can reduce or blocked the probability of entrance of fiber in to the milk resulted in less fiber content due to less pressure within the pores during soy milk extraction. Balogun and Fetuga, (1986) revealed that the low crude fiber is nutritionally

appreciated because it traps less proteins and carbohydrates.

Ash content: The ash content of soy milk produced from soybean soaked at 40°C was significantly ($p < 0.05$) higher (8.30%) than that of the milk produced after soaking the soybean at 25°C (6.62%) (Table 4b). The value increased with increasing in soaking temperature attaining the highest value at the highest soaking temperature due to expansion of the tissue. This value was higher than 4.83% and 5.02% observed by Adebayo, *et al.*, (2008). The difference was probably due to processing and variety difference (Saikia *et al.*, 1998).

Soybean subjected to 16 hrs soaking had significantly ($p < 0.05$) higher ash value (8.13 %) than other two soaking times in soymilk. Whereas the remaining two soaking times (8 and 12 hrs) had not showed significant differences between each other. The result showed the ash content of soymilk was increased with increasing the soaking time. This might be due to prolonged exposure of the soybean to soaking which increased degradation of tissues.

Soymilk ash content was significantly ($p < 0.05$) influenced by soybean soaking time and temperature (Table 4b). Accordingly, higher ash value (10.08%) was measured for soaking time of 16 hrs at 40°C. This result agreed with the findings of (Orhevba, 2010) who reported the ash content of soy milk from 4.5 to 10.5%. Ash contents in this study also agreed with the findings of Liu, (1997) that found 6 to 9.2% but some difference observed because of the higher temperature i.e. (50°C) used during soaking. The presence of ash in the soymilk produced, gave an indication of the presence of inorganic minerals in it. But among other treatments of 8 hrs soaking at 40°C, 12 hrs soaking at 25°C and 12 hrs soaking at 40°C, ash content of soy milk were not significantly varied.

Total carbohydrate: The total carbohydrate of soy milk was significantly affected ($p < 0.05$) by temperature. The highest total carbohydrate content (10.65%) was observed in the soy milk sample extracted from soybean soaked at 25°C and the lowest (9.87%) was for soy milk sample extracted from soybean soaked at 40°C (Table 4b). This value was higher as compared to 9.22% observed by Bhumiratana, (1978). The present result was lower than 11.3% reported by Salunkhe and Kadam (1989). This report indicated that processing will affect the total carbohydrate content of soy milk. The result obtained in the present study had agreed with Wilkens and Hackler, (1969) in which a reduced carbohydrate content and increase in lipid content was reported with soaking effect. The longer soaking at higher temperature had reduced solid yield. In other work (Bhumiratana, 1978) soaking temperature above 45°C with longer soaking time had resulted in a large decrease in the total solids and carbohydrate. The effect of short

duration of soaking was also predominantly because of leaching of water soluble carbohydrate. Processing has different effect on different food products. In soymilk, crude protein, ash and fat were increased whereas the total carbohydrate and crude fiber were decreased from the original value.

Soymilk that obtained from soybean soaked for 8 hrs resulted in significantly ($p < 0.05$) higher value of total carbohydrate (11.96%) than those treated with 12 and 16 hrs soaking time (Table 4b). The later value was not significantly different between each other. It showed that when the soaking time increased, a decrease in the total carbohydrate content. This result in agreement with Bhumiratana, (1978) reported that the major effect of soaking on composition was to reduce total carbohydrate and increase lipid content. Longer soak reduce solid yield.

Soymilk carbohydrate was significantly ($p < 0.05$) affected by interaction effects of time and temperature. Accordingly, carbohydrate content (13.81%) was higher for soybean soaked for 8 hrs at 25°C (Table 4b). However, there was no significant difference between 12 and 16 hrs soaking at both temperatures. The result showed a decreasing trend as soaking time increased. This result was supported by Wilkens and Hackler, (1969) who reported that the total solids decreased with both increasing soaking time and temperatures for dehulled beans. The effect of short duration soaking was predominantly leaching of water soluble carbohydrate. On the average, carbohydrate accounted 60% of the solids contained in the soaking water.

pH: Soy milk pH was not affected by temperature. The value was 6.67 (Table 4b). This was similar to 6.74 reported by Bahareh (2009). The present value was in the range reported by Trongpanich, *et.al* (1988) who stated that the proper range of pH of soymilk was (6.6 to 6.8). The same author reported that lowering the pH (< 6.6) would result into protein precipitation and an increase in the pH (> 6.8) would affect the milk flavor. Similarly, the time was not significant on the pH of soy milk as soy milk produced after soaking 8, 12 and 16 hrs had 6.71, 6.67 and 6.64 pH of soymilk, respectively.

As shown in Table 5b the pH of the soy milk not significantly influenced by interaction of soaking time and temperature. Generally the pH was reduced as soaking time extended from 8 to 16 hrs although some irregularity was observed.

d) *The Effect of Soaking Temperature and Time on Sensory Attributes of Soybean Milk*

i. *Color*

Temperature was significantly different ($p < 0.05$) on color acceptability of soy milk (Table 6). At 40°C soaking temperature soy milk color was found to have significantly higher score (6.71) than at 25°C. The

product obtained at 40°C was more accepted by consumer panelists. The colors of the soybean milk that obtained from the two soaking temperatures were above average based on the scale used on the evaluation sheet. Heat treatment (blanching and hot water soaking) were known to reduce lipoxygenase enzyme which deteriorated color and thereby keeping the natural color of the soy milk. According to Cauvain and Young (2001) soybean contained lipoxygenase enzyme, which oxidized carotenoid and chlorophyll pigments. Muller, (1988) also reported that soybean lipoxygenase inactivated by blanching. Since soybean was blanched prior to soaking, this might be blocked the activation of enzyme or color oxidation.

Soy milk color was significantly affected by soybean soaking time (Table 6). The soymilk prepared by soaking for 8 hrs was found to have significantly ($p < 0.05$) higher (6.82) than 12 and 16 hrs soaking time. Between the products obtained at 12 hrs and 16 hrs significant differences was not observed. The former soy milk color was perceived as more accepted by consumer panelists as compared to the later. Soy milk color acceptance had increased when soaking time decreases from 16 hrs soaking to 8 hrs soaking. Longer soaking time is shown to have slightly reducing the color acceptance of the soy milk; it becomes yellowish color with increasing soaking time.

The interaction effect of soaking time and temperature on sensory quality of soy milk color is presented in Table 6. Soy milk which was made from soaked soybean at 40°C soaking temperature with 8 hrs soaking time resulted in highest sensory score (7.00) of color than other treatments, there was no significant difference among others. But color acceptance was close to "like moderately" based on the scale and this indicated that panelists had accepted the color of all soymilk. The product was more acceptable in color at lower soaking time with higher soaking temperature. According to evaluator perception, color was major parameter in sensory attribute of soymilk. The highest sensory score of milk color might be due to optimum soaking temperature and time in combination with proper blanching that prevented from oxidation of color pigments and which gave the product more acceptability.

Table 6: Effect of soaking temperature and time on sensory quality attributes of soybean milk

| Variables | | Color | Flavor | Odor | Overall acc. |
|--|-------------|--------------|--------------|--------------|--------------|
| Time | Temperature | | | | |
| Main effect of soaking temperature (25 and 40°C) and time (8, 12 and 16 h) | | | | | |
| | 25 | 6.55±0.62b | 6.31±0.64a | 6.37±0.75a | 6.50±0.50b |
| | 40 | 6.71±0.46a | 6.37±0.55a | 6.44±0.64a | 6.67±0.50a |
| 8 | | 6.82±0.39a | 6.61±0.49a | 6.84±0.66a | 6.90±0.30a |
| 12 | | 6.55±0.67b | 6.53±0.50a | 6.57±0.50b | 6.70±0.46b |
| 16 | | 6.52±0.50b | 5.88±0.52b | 5.80±0.45c | 6.16±0.42c |
| Interaction effect (soaking time and temperature) | | | | | |
| 8 | 25 | 6.64 ± 0.48b | 6.54±0.50ab | 6.72 ± 0.90b | 6.80 ± 0.40b |
| 8 | 40 | 7.00 ± 0.00a | 6.68 ± 0.47a | 6.96 ± 0.20a | 7.00 ± 0.00a |
| 12 | 25 | 6.48 ± 0.81b | 6.64 ± 0.48a | 6.96 ± 0.20a | 6.66 ± 0.47b |
| 12 | 40 | 6.62 ± 0.49b | 6.42 ± 0.50b | 6.58 ± 0.50b | 6.74 ± 0.44b |

CV=coefficient of variance, Values followed by different letters indicate a significant difference ($p<0.05$), Mean \pm SD; Number of panelist = 50.

The attractive color could be probably due to modern extraction material (blender) which was protected entrance of unwanted material into the milk and allowed the okara to remain on the sieve; this may keep the natural color of the milk. According to Trongpanich, et al. (1988) in the traditional way of soy milk production, the slurry was cooked since it was well recognized technique for improving the flavor. However, even slight over cooking resulted in strong sulfur and burnt smell as well as dark color in the appearance of soymilk.

ii. Flavor

There was no significant difference between the two soaking temperatures on flavor acceptance of soy milk (Table 6). All products had good flavors and this might be due to optimum blanching which stopped the enzymatic activity that helped to produce acceptable flavor. The result indicated that blanching and soaking were examined the composition of soy milk extracted with hot water prevent development of rancid flavor. Guy (2001) reported that unprocessed soybeans have off-flavor. Off-flavors are often generated through enzymatic lipid oxidation. The enzymes involved in lipid oxidation are lipoxygenases. Hymowitz and Polmer (2004) also described that during soymilk production; distinctive flavors were developed that were variously described as beany, rancid, and bitter. The flavor was due to the action of native soybean enzyme.

The effect of soaking time on flavor is presented in Table 6. The soy milk prepared from soybean soaked for 16 hrs had significantly ($p<0.05$) lower score (5.88) than others. However, there was no significant difference between 8 hrs and 12 hrs soaking time. Generally, as the soaking time increased from 8 to 16 hrs the flavor acceptance score decreased. Optimum

soaking time avoided most off-flavor compounds resulting in good flavor acceptance. Longer soaking time gave slight bitterness to the product. According to Perkins, (1989) during heat treatment, time was critical to obtain optimum results with the least protein denaturation.

Interaction effect of soaking time and temperature was significantly affected the soy milk flavor (Table 6). Soy milk flavor was significantly lower (5.74) which were obtained from soaked soybean for 16 hrs at 25oC than other treatments (8 hrs soaking at 40oC, 12 hrs at 25oC, and 8 hrs at 25oC soaking). The entire flavor acceptance score were above "moderately like" except few, which are very close to "moderately like" indicating that all the products had acceptable flavor by the panelists.

The result showed that the beany flavor of soy milk was reduced to greater extent by blanching and soaking. According to Taylor, (1996) flavor was considered as a combination of aroma, taste and trigeminal perceptions from stimulation of the mouth and nasal area. Wilkens *et al.* (1967) had reported that heat treatment could reduce the off flavor in soy milk. Wilkens and Hackler, (1969) also reported that range of processing conditions were known to influence the composition and yield of soybean milk and among these blanching and soaking of soybean seed were known to prevent the formation of rancid flavor.

iii. Odor

Soy milk odor was not affected by soaking temperature. Unlike temperature, soaking time had significant influence ($p<0.05$) on soymilk odor (Table 6). Significantly higher score (6.84) was observed at 8 hrs soaking time. The result showed that as the soaking time extended from 8 hrs to 16 hrs, the odor acceptability

of soymilk was reduced. Based on the scale (seven point hedonic scale) considered in this study for sensory evaluation, longer soaking time were not required for acceptable soy milk odor.

The combined effect of soaking time and temperature was significant ($p < 0.05$) on odor of soy milk (Table 6). Soybean subjected to 40°C and 25°C soaking temperature for 8 hrs and 12 hrs, respectively, resulted in more acceptable odor with value of 6.96 compared to soymilk odor obtained from soybean that was treated at 25°C and 40°C soaking temperatures for 16 hrs. The result showed that the former soymilk was better accepted based on the scale used in the evaluation sheet.

iv. Overall acceptability

The overall acceptability of soy milk was influenced by soaking temperature which expressed as the cumulative effects of the selected parameters (Table 6). More accepted product was obtained from soybean treated at 40°C. Soy milk made from soaked soybean at 25°C was found to have lower acceptance. This showed that soaking temperature significantly affected the overall acceptability of soy milk. The score of overall acceptability tended to decrease with decreasing soaking temperature due to the occurrence of some off flavoring compounds.

Over all acceptability of soy milk was significantly ($p < 0.05$) reduced with increasing soaking time. Soybean treated for 8, 12, and 16 hrs soaking period scored 6.90, 6.70, and 6.16, respectively, (Table 6) The decreased in overall acceptability occurred when the product soaked for longer time the compounds leached into the water and reabsorbed by the grain. The highest acceptability soy milk produced from 8 hrs soaking. Moreover, the overall acceptability were above moderate indicating higher level of the overall quality of the milk.

The interaction effects of soaking time and temperature on sensory evaluation of soy milk regarding the overall acceptance is presented in Table 6. Soy milk prepared from soybean soaked for 8 hrs at 40°C were found to have significantly ($p < 0.05$) higher (7.00) overall acceptance than others. Soymilk prepared from soybean soaked at 25°C for 16 hrs was found to be the least acceptable. The overall acceptability was based on consumer panelist experience as a personal appraisal. Hot water soaking is extremely important for the overall acceptability because it changed properties of soybean. Soaking for relatively hot water with shorter soaking time gives good acceptance to the soy milk than lower temperature and longer soaking time.

IV. SUMMARY AND CONCLUSIONS

a) Summary

The present study was conducted to investigate the effects of soaking time and temperature on proximate composition and sensory quality of soy milk.

The experiment was comprised two soaking temperature (25°C and 40°C) and three soaking times (8, 12, and 16 hrs), total six treatments for each (soy flour and milk) and laid in CRD with three replications. Sensory evaluations were conducted on soy milk to evaluate color, flavor, odor and overall acceptability. A total of 50 male and female consumer panelists were involved in the evaluation.

Soaking time and temperature were found to have significant effects on the proximate composition. Flour protein, fat and total carbohydrate were significantly ($p < 0.05$) higher (44.26%, 21.98%, 27.12%) for soybean soaking time of 16 hrs at 40°C, 8 hrs at 40°C and 12 hrs at 25°C as compared to raw soybean flour 44%, 20.80% and 20.0%, respectively. However, crude fiber (3.77%) and ash (4.47%) were less in soybean flour. Soy milk protein, fat and total carbohydrate were significantly ($p < 0.05$) higher (52.35%, 23.98%, and 13.81%) for soybean soaking time 16 hrs at 25°C, 16 hrs at 25°C, and 8 hrs at 25°C, respectively. Compared to other treatment crude fiber value were low (0.03%) at 8 and 12 hrs soaking time with 40°C soaking temperature.

Soaking temperature and time had significant effect on soy milk quality. The soybean milk quality was evaluated by sensory panelists for color, flavor, odor and overall acceptability. The most accepted milk was obtained from soy bean soaked for 8 hrs at 40°C in all parameters. The sensory mean score for color, flavor, odor and overall acceptability were 7.00, 6.68, 6.96 and 7.00, respectively on seven point hedonic scale.

b) Conclusion

The current study revealed that soaking time and temperature had increased protein, fat and total carbohydrate whereas crude fiber and ash were lower than raw soybean flour. In soy milk, protein, fat and ash were increased while total carbohydrate and crude fiber were reduced. It was concluded that the two products needed different processing conditions to improve their protein properties. Optimum processing time and temperature was important for increasing nutrient content. Processing included soaking and dehulling. The most accepted soybean flour and milk was produced from soy bean soaked at 8 hrs with 40°C in all parameters.

c) Recommendations

In this experiment to recommend optimum processing methods for soy flour and soy milk, two important points were considered: treatments which increased or maintained the proximate composition and sensory quality. Accordingly, soaking of soybean for 8 hrs at 40°C is recommended for soy flour production. Based on the result, at this time and temperature the nutritional compositions were positively affected. Although proximate composition was relatively not high for soaking of 8 hrs at 40°C as compared to other

treatments, this optimum processing time and temperature is recommended for soy milk due to sensory quality.

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The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in a research paper?" Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

PREPARATION OF ELETRONIC FIGURES FOR PUBLICATION

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

TIPS FOR WRITING A GOOD QUALITY SCIENCE FRONTIER RESEARCH PAPER

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.



20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

THE ADMINISTRATION RULES

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Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

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Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.



CRITERION FOR GRADING A RESEARCH PAPER (COMPILATION)
BY GLOBAL JOURNALS

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

| Topics | Grades | | |
|-------------------------------|--|---|--|
| | A-B | C-D | E-F |
| <i>Abstract</i> | Clear and concise with appropriate content, Correct format. 200 words or below | Unclear summary and no specific data, Incorrect form Above 200 words | No specific data with ambiguous information Above 250 words |
| <i>Introduction</i> | Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited | Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter | Out of place depth and content, hazy format |
| <i>Methods and Procedures</i> | Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads | Difficult to comprehend with embarrassed text, too much explanation but completed | Incorrect and unorganized structure with hazy meaning |
| <i>Result</i> | Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake | Complete and embarrassed text, difficult to comprehend | Irregular format with wrong facts and figures |
| <i>Discussion</i> | Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited | Wordy, unclear conclusion, spurious | Conclusion is not cited, unorganized, difficult to comprehend |
| <i>References</i> | Complete and correct format, well organized | Beside the point, Incomplete | Wrong format and structuring |



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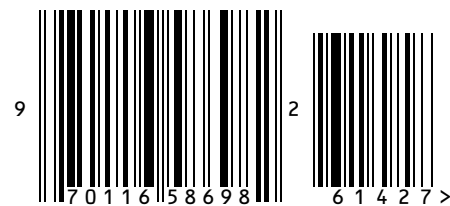
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