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EFFECT OF INFRA-RED AND ULTRAVIOLET RADIATION ON STERILIZATION AND TRYPSIN INHIBITOR DEACTIVATION OF COWPEA SEEDS

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Abstract

Experiments were carried out through summer season 2019 in the Rice Mechanization centre at Meet El-Deeba, Kafr El-Sheikh Governorate to study the effect of infra-red radiation and ultraviolet radiation on protein, trypsin inhibitor and total microbial count of cowpea seeds as pre- conditioning methods prior to storage process. Five exposure times of (3-6-9-12-15 min) and five irradiation intensity (804.255, 882.67, 964.74, 1,050.45, 1,139.8 W/m2) were used for infra-red treatments. For ultraviolet treatment four exposure times (10-20-30-40min) and three irradiation intensity (7.077 – 3.538 – 2.359 mW/cm2) were used. For the IR conditioning method, irradiation intensity of 882.67 W/m2 at exposure time of 15 min is recommended. This level of radiation intensity and exposure time, showed total microbial count of 2.3 Log CFU/g., protein content 28.88 %, trypsin inhibitor 1.148 TIU/mg and moisture content 8.13 % of cowpea seeds. Meanwhile the irradiation intensity of 3.538 mW/cm2 at exposure time of 40 mins is recommended for UVC irradiation pre-treatment to get total microbial count 2 Log CFU/g., protein content 28.15%, trypsin inhibitor 0.57 TIU/mg and moisture content 10.95%.

Key words: *cowpea, infra-red radiation, ultraviolet radiation, protein, trypsin inhibitor, total microbial count*

INTRODUCTION

Cowpea seeds (*Vigna unguiculata*) is a legume that is used in several parts of the world as a high- value plant protein source. Cowpea is an necessary nutritious food in the human diet because of its high protein and carbohydrate content, low fat content, and complement amino acid pattern to cereal grains [1]. Cowpea has gained more interest from researchers and consumers all over the world because of its exerted health useful properties excluding anti-hyperlipidaemia, anti-diabetic, anti-inflammatory, anti-cancer and antihypertensive properties [7]. Cowpea is cultivated through the world on an estimated 14.5 million ha of land planted every year and the total yearly production is 6.2 million metric tons and in Egypt is 1,853 hectares with production 7,180 tons [11].

Cowpea seeds are similar to other pulses in terms of nutrition, with a low fat content and a high total protein content. Cowpea is a nutrient-dense food with a low energy content. Legumes have higher protein content than cereals, ranging from 17 to 30 percent. Since the protein content of legumes is roughly equivalent to that of some meats, they are regarded as the meat of poor people [9]. Moreover, the Cowpea protein is high in amino acids corresponding tryptophan and lysine, making it a perfect supplement to carbohydrates tubers and cereals. Cowpea protein, on the other hand, is low in cysteine and methionine as compared to proteins of animal [6]. Owing to the existence of anti-nutritional factors, protein digestibility in pulses varies, but it is usually lower than in cereals and proteins of animal [21] [25] legumes have much higher concentrations of antinutrtional than cereals, trypsin inhibitor activity, is considered anti-nutritional factor that has a strong relationship with protein digestibility.

The infrared region of the electromagnetic spectrum lies between visible light and microwaves, with wavelengths ranging from (0.5 to 100 μm). Near-IR (NIR) rays have wavelengths ranging from (0.75 to 1.4 μm) at temperatures below 400°C, mid-IR (MIR) rays have wavelengths ranging from (1.4 to 3 μm) at temperatures between 400 and 1,000°C, and far-IR (FIR) rays have wavelengths ranging from (1.4 to 3 μm) at temperatures above 1,000°C [22, 23].Compared to conventionally processed mung bean (*Phaseolus aereus*) beans, the effect of infrared processing on crude protein and trypsin inhibitor resulted in a significant decrease of trypsin inhibitor although the crude protein did not change significantly [20].

Among X-rays (100 nm) and visible light (400 nm), UV-light spans a wide wavelength range in the nonionizing field of the electromagnetic spectrum. UV light is divided into three wavelength bands: UV-A, UV-B, and UV-C [5].

UV light is a physical method that has many benefits, including the absence of by-products that may alter the characteristics of the food, the absence of chemical residues, and the fact that it is a dry, simple and cold process, efficient, and low-cost process as compared to other sterilization methods. It also does not emit any form of ionizing radiation [12].

UVC light can also be used to inactivate a variety of organisms; it has been used as a disinfection medium in the pharmaceutical, electronic, and aquaculture industries for many years. Low-pressure mercury vapour germicidal lamps emit monochromatic UV light (254 nm) [3]. The UVC radiation absorbed by DNA has the potential to halt cell growth and cause cell death. UVC light absorbed by DNA induces a physical change of electrons, resulting in DNA bond breaking, cell death, or a delay in reproduction [3, 15, 26].

The current study aims at testing and evaluating two different conditioning methods (FIR-UVC radiation) for cowpea seeds sterilization and trypsin inhibitor deactivation.

MATERIALS AND METHODS

Materials - Cowpea seeds

The cowpea seeds var. (Dokki 126) were used for this experiment. The tested samples were obtained from EL Aiatt, Giza Governorate, Egypt. The pre-treating methods were conducted as follows:

Infrared heating unit

The infrared heating unit shown in Fig.1 was used as thermal treatment for the experimental work. The unit consists of a rotary cylinder (0.6 m diameter and 0.2 m long) made of 1 mm galvanized iron sheet enclosed by a fixed insulated cylinder (0.8 m diameter and 0.3 long), one side of the rotary cylinder connected to a driving mechanism consists of 0.15 m diameter steel flange fixed to the side cover of the rotary cylinder and welded to a steel bar riding with a heavy duty ball bearing. A 0.5 kW low speed motor with different sizes of bullies was used for power supply and speed control of the rotary cylinder. For heating and temperature control of the infrared heating unit, two ceramic infrared heaters (1 kW/each) were fixed over two iron blades and assembled into the centre iron bar of the rotary cylinder facing the cowpea bulk. For controlling the distance between the ceramic heaters and the cowpea surface two screw rods were welded to the iron blades to allow movement of the heaters up and down.

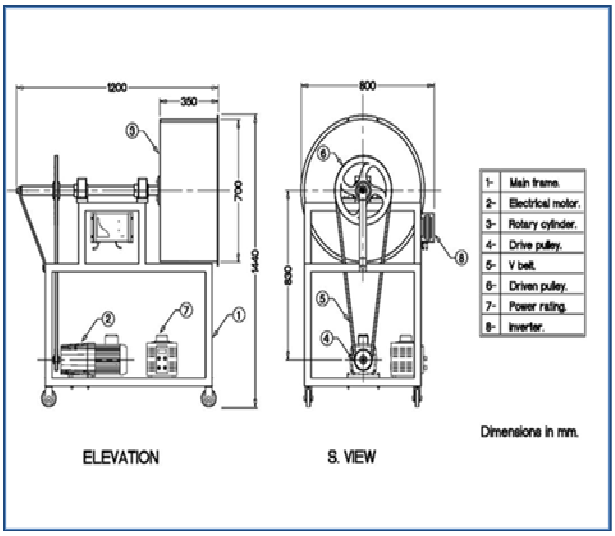
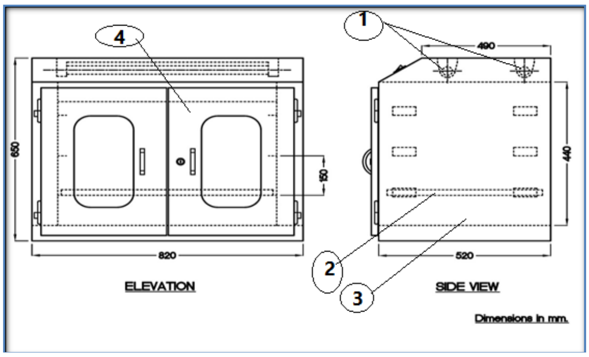


Fig. 1. The infrared heating experimental unit
Source: Author's own illustration.

Ultraviolet treatment unit

Ultraviolet radiation treatment of Cowpea was conducted using an irradiator consisting of two UVC lamps (4136 G36T6-20W - 254 nm), the length of each lamp is 60cm as shown in Fig. 2. The unit body was made of metal sheet plated with electrostatic substances (820 mm length and 520 mm width), the inside surface of the unit was made of stainless steel 304 with a door made of poly carbonate for protection from uv-c radiation. The operation of the lamps was controlled by a timer which was set for various exposure times of 10, 20, 30 and 40 min. The cowpea seeds were placed horizontally in a uniform manner on a portable shelf and set in various distances from the ultraviolet source (150- 300 - 450 mm). The irradiation power was set at 10 mW cm^{-2}.



Legend:

NO	Part name
1	2 Lamps UVC 20W/once (254nm)
2	Portable shelf
3	The body of UVC unit
4	The door of the unit

Fig. 2. Experimental setup for UV-C radiation treatment.
Source: Author's own illustration.

Experimental method

(1) Infra-red pre-treatment

The rotary cylinder was operated continuously at the required level of irradiation intensity that controlled by powering rate and the treated sample was discharged through the removable sector of the rotary cylinder bottom after the tested exposure time. At the end of each treatment, the treated sample was left in a wooden frame until reaching room temperature. Moisture content, protein content, trypsin inhibitor and total microbial count were measured for each studied treatment.

(2) Ultraviolet pre-treatment

The UVC unit was operated at the recommended level of irradiation intensity and exposure time. After the tested exposure time, the irradiated samples were used for measuring protein content, trypsin inhibitor and total microbial count

Measurements:

-Determination of moisture content

The moisture content was determined by the standard oven method at 105°C for 24 hours [2].

The moisture content was calculated at wet basis (w.b. %) as following:

$$Mwb = [(mw - md) / mw] \times 100$$

where:
Mwb: Moisture content, %.
mw: Wet mass, g.
md: Dry mass, g.

-Bulk temperature of treated seeds

Data recorder model YEW 3057 was used to measure bulk temperature of cowpea seeds with thermocouple type T.

- Determination of total protein content

The total nitrogen was determined by using micro Kjeldahel method according to the method of A.O.A.C. (1990). Total protein was calculated by multiplying the total nitrogen by 5.57.

-Trypsin inhibitor (TIU/mg)

The trypsin inhibitor (TI) activity was calculated by incubating 50 µl of crude extract with 20 µl of commercial bovine trypsin (1 mg mL-1) for 15 minutes at 37°C, as defined by [13].

-Total microbial colony count (microbiological analysis), cfu/g.

Total microbial count activity was determined following the methodology; about 25 g from the samples was transferred in to a stomacher bag (Seward, London, UK), and homogenized with 225 ml of sterile salin peptone water (SPW:1g/1 peptone, 8.5g/l sodium chloride) for 3 min. A 10- fold serial dilution was made from each sample and used for quantitative microbiological examinations after treatment with Serial dilutions of sterile saline peptone water with samples was prepared, and duplicate of 1 ml samples of appropriate dilutions were poured on agar plates. Total bacterial count (TBC) was enumerated on plate count agar (Merck, 1.05463) at 30^0C for 48 h. All plates were examined for typical colony types and morphological characteristics associated to each culture medium.

RESULTS AND DISCUSSIONS

Infra-red pre-treatment:
Seeds moisture content

The effect of different irradiation intensity and exposure time on moisture content of seeds is shown in Fig. 3. The initial cowpea moisture content was 10.95 % w.b and the final moisture contents after the treating process were (8.86, 8.13, 5.72, 5.37, 5.28 % w.b) at different levels of irradiation intensity of (804.255, 882.67, 964.74, 1,050.45, 1,139.8 W/m^2.) respectively.

It was obvious that the moisture removal increased with the increase of exposure heating time under all levels of irradiation intensity. This condition may be due to the fact that, when the seeds are infra-red heated; the penetration of the infrared rays into the material causes the water molecules to vibrate which results in rapid internal heating of seeds moisture which arises the water vapour pressure inside the seeds with a corresponding loss of water to the surrounding air. The removal rate of water from seeds layers was higher during the initial stage of infrared heating and starts to decline with the heating time as mentioned by [10].

Change of bulk temperature of cowpea seeds at different irradiation intensity and exposure time

Cowpea bulk temperature as related to heating time is presented in Fig. 4. As shown in the figure, the temperature of heat treated samples depending on heating time and irradiation intensity. The bulk temperature of cowpea seeds approached 56.9°C, 69.9°C, 90.1°C, 101.3°C and 111.4°C at the maximum exposure time of 3 min and approached 89.5°C, 97.5°C, 111°C, 121.9°C and 130.5°C, at the maximum exposure time of 15 min for irradiation intensity levels of 804.25, 882.67, 964.74, 1,050.455 and 1,139.81 W/m^2 respectively.

The figure also shows that, seeds bulk temperature was lower during the early stage of heating and it was increased with longer exposure time. This means that during the initial stage of heating the temperature difference between the bulk temperature of cowpea and the heating temperature was relatively high which causes higher rate of heat transfer from the heating source (Infra-red) to the cowpea seed. This causing an increasing rate of bulk temperature during this early stage of heating. However, as the difference between the temperature of heating source and cowpea seeds decreased with increasing the exposure time the rate of heat transfer is also decreased.

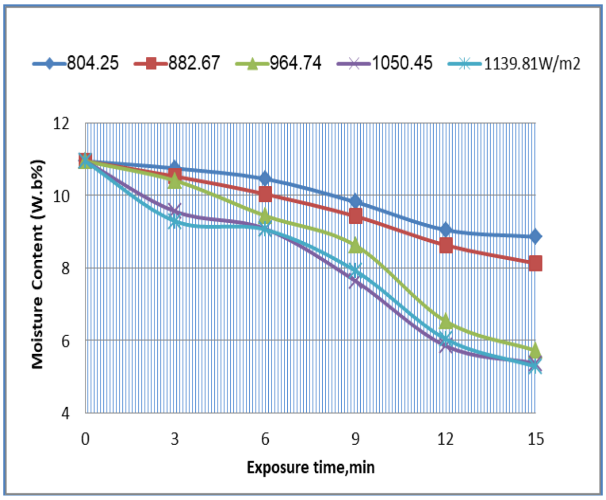


Fig. 3. Change of seeds moisture content at different irradiation intensity and exposure time.
Source: Authors' determination.

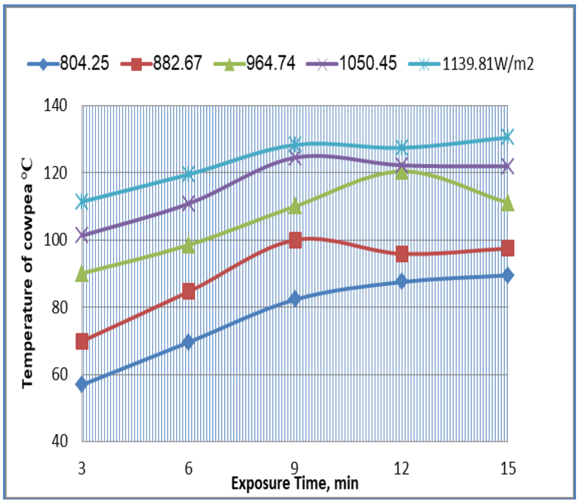


Fig. 4. Change of bulk temperature of cowpea seeds at different irradiation intensity and exposure time.
Source: Authors' determination.

Protein content on cowpea seeds

The effect of different irradiation intensity and exposure time on protein content of cowpea seeds is illustrated in Fig. 5. The protein content was calculated by total nitrogen in cowpea seeds The protein content of raw seeds was 30.29% and reduced to 29.62%, 28.88%, 27.89 %, 25.33% and 23.52% at the maximum exposure time of 15 mins and reduced to 30.2%, 30.1%, 29.94%, 28.71% and 28.14% at the min exposure time of 3 mins for irradiation intensity of 804.25, 882.67, 964.74, 1,050.455 and 1,139.81 W/m^2 respectively.

When legume seeds are infrared heated, the protein becomes more vulnerable to denaturation and aggregation, lowering its solubility [17, 19]. As a result, the amount of total protein decreases. Meanwhile, when legume seeds are heated with infrared radiation at temperature of 150°C, extreme protein denaturation occurred [27].

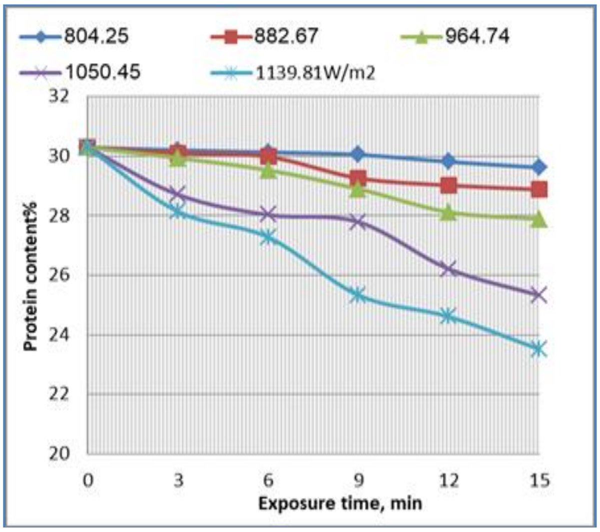


Fig. 5. Effect of different irradiation intensity and exposure time on Protein content of cowpea seeds.
Source: Authors' determination.

Trypsin inhibitor of cowpea seed

Figure 6 presents the effect of different irradiation intensity and exposure time on trypsin inhibitor of cowpea seeds. Trypsin inhibitor of raw seeds was 2.214 TIU/mg and reduced to 2.209, 2.112, 2.103, 2.103, 2.009 and 1.734 TIU/mg at the minimum exposure time of 3 min. while, it was and reduced to 1.734, 1.148, 1.142, 1.139 and 1.135 TIU/mg at the exposure time of 15 min for irradiation intensity 804.25, 882.67, 964.74, 1,050.455 and 1,139.81 W/m^2 respectively. When comparing the level of trypsin inhibitor for the non-treated cowpea seeds (2.214 TIU/mg) with that treated with infra-red, it can be said that, heat treatment with infrared decreased the trypsin inhibitor by 0.22% to 48.73% depending upon the level of radiation intensity and the exposure time. The above-mentioned results could be attributed to the heat denaturation of protein. Also, it has causes enhancement of protein digestibility mainly through the inactivation of thermo labile ant nutritional factors such as trypsin [4].

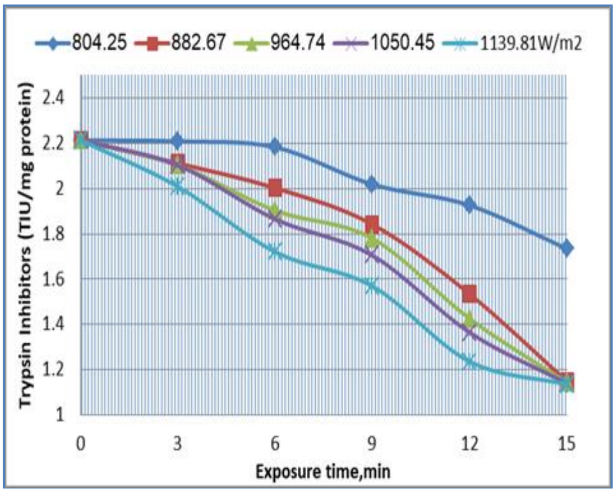


Fig. 6. Effect of different irradiation intensity and exposure time on trypsin inhibitor of cowpea seeds
Source: Authors' determination.

Total Microbial count (Log10 CFU/g) of cowpea seeds

Figure 7 presents the effect of different irradiation intensity and exposure time on total microbial count of cowpea seeds. As shown in the table, the total microbial count (TMC) reduced from 4.7 to 4.6 (Log10 CFU/g) at the minimum irradiation intensity of 804.255 W/m^2 and exposure time of 3 mins, while it was reduced from 4.7 to 2.3 at the maximum irradiation intensity of 1,139.80 W/m^2 and exposure time of 15 mins.

The above mentioned results revealed that, absorbing energy from infrared radiation causes thermal effects, as it raises the temperature of the cowpea seeds to a level enough to stop the activity of microorganisms [24]. Meanwhile, the results did not detect any fungal count (TFC) for both heat treated and non-treated samples.

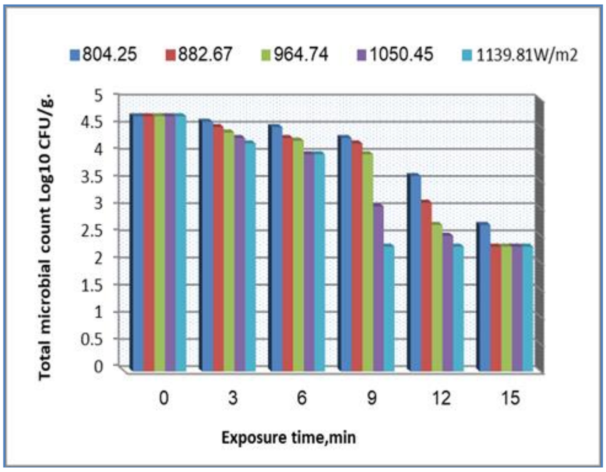


Fig. 7. Effect of different irradiation intensity and exposure time on total microbial count (Log10 CFU/g) of Cowpea seeds.
Source: Authors' determination.

Ultra-violet pre-treatment:
Protein content of cowpea seeds

The effect of different irradiation intensity and exposure time on protein content of cowpea seeds was illustrated in Fig. 8. At the highest radiation intensity of 7.077 mW/cm^2 and the maximum exposure time of 40 mins, the total protein content decreased from 30.29% to 24.92% leading to a maximum diminution of 17.7% for the initial protein.

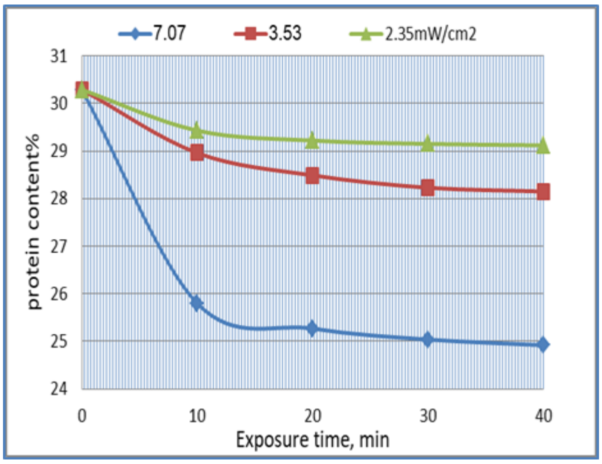


Fig.8. Effect of different irradiation intensity and exposure time on protein content of cowpea seed.
Source: Authors' determination.

The lowest radiation intensity of 2.359 mW/cm^2 decreased the total protein content from 30.29% to 29.43 % at the minimum exposure time of 10 mins leading to the diminution of 2.84% of the initial protein. The reduction in total protein content upon UV radiation can be imputed to the formation of nitrogen-free radicals and released them from cowpea seeds in the form of ammonia or Nitrogen oxides through the irradiation process with UVC. The aromatic residue like tryptophan, tyrosine, or cysteine amino acids in protein was absorbed by UVC radiation [19]. UVC radiation allows proteins and amino acids to degrade and create ammonia gas, with non-peptide nitrogen-containing amino acids like histidine and tryptophan leading to the release gas of ammonia [14, 18].

Trypsin inhibitor of cowpea seeds

The effect of different UVC irradiation intensity and exposure time on trypsin inhibitor of cowpea seeds is illustrated in Fig. 9. The level of trypsin inhibitor in the raw cowpea seeds was 2.214 TIU/mg. As shown in the results, the highest UVC radiation intensity of 7.077 mW/cm^2 at maximum exposure time of 40 mins, reduced the activity of trypsin inhibitor to 0.694 TIU/mg. However, the lowest irradiation intensity of UVC of 2.359 mW/cm^2 at the minimum exposure time of 10 mins, reduced the trypsin inhibitor activity to 1.133 TIU/mg.

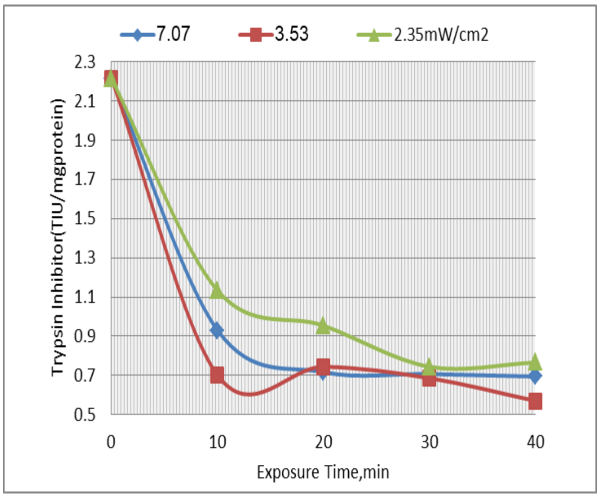


Fig. 9. Effect of different irradiation intensity and exposure time on trypsin inhibitor of cowpea seeds.
Source: Authors' determination.

From Fig. 9, it can be concluded that, the most effective irradiation intensity on trypsin inhibitor was 3.538 mW/cm^2 at exposure time of 40 mins as it reduced activity by 74.25%.

This mean that, UVC radiations is a very effective treatment for elimination of antinutritional elements (trypsin inhibitors), as mentioned by [8].

Total microbial count (Log10 CFU/g)

As shown in Fig. 10, the total microbial count decreased from 4.7 to 2 (Log10 CFU/g) at the highest irradiation intensity of 7.077 mW/cm^2 and the maximum exposure time of 40 mins. While it was reduced from 4.7 to 2.4 (Log10 CFU/g) at the minimum irradiation intensity of 2.359 mW/cm^2 at maximum exposure time of 40 mins. The germicidal properties of ultraviolet irradiation are due to the DNA absorbing of UV light, causing crosslinking between neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand [16]. The results also show that, Fungi count in cowpea seeds was not confirmed whether for treated or non-treated samples.

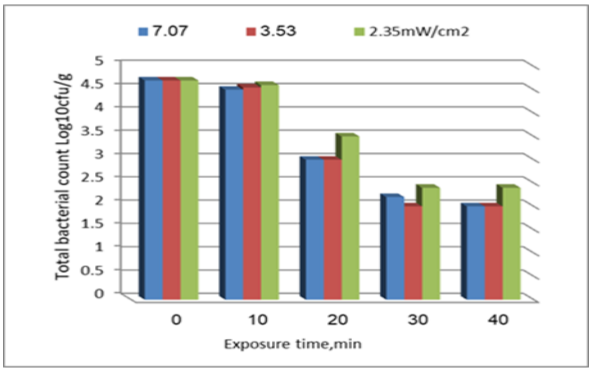


Fig. 10. Effect of different irradiation intensity and exposure time on total microbial count (Log10 CFU/g) of cowpea seeds.
Source: Authors' determination.

CONCLUSIONS

In general, the analysis of results showed that, For infra-red pre-treatment the irradiation intensity of 882.67 W/m^2 at exposure time of 15 mins is recommended. At this level of radiation intensity and exposure time, the total microbial count was 2.3 Log CFU/g., protein content 28.88 %, trypsin inhibitor 1.148 TIU/mg and moisture content 8.13 % w.b. Meanwhile, for the UVC pre-treatment, irradiation intensity of 3.538 mW/cm2 and exposure time of 40 mins is recommended to get total microbial count of 2 Log CFU/g., protein content 28.15%, trypsin inhibitor 0.57 TIU/mg and moisture content 10.95%.

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