

Steam-Microwave on Reduction of Microbial Level of Red Pepper and Compared with Gamma Irradiation

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ABSTRACT

Red pepper is utilized as a single flavor or in combination with other spices. This spice contains capsaicin, Carotenoid pigment and high Ascorbic acid level which have proper health and Anti-cancer effects on it. Normally this product is replete with microbial load and pathogens prior to applying any decontamination methods that could threaten human life. So creating a new approach for microbial decontamination of whole dry red pepper is obligatory, which is applying steam and microwave together. Here, saturated steam and microwave heat is used for 10 minutes with 720 watts and for 90 seconds respectively, while microbial load, enzymatic activities, moisture content and color alternation were monitored. Finally it was observed that processed red pepper contains the minimal microbial load and lacks any enzymatic activities, even in comparison with applying gamma irradiation with 5K Gy in which enzyme activity was noticeably seen.

Keywords : Red pepper, Irradiation, Microbial load, Microwave, Capsaicin

I. INTRODUCTION

Red Pepper is planted all over the world and is used both in fresh and dry forms. This product basically is used for creation of desired red color along with a sharp taste, it has been known that (Kim et al. 2014; Rico et al. 2010). Red Pepper contains a significant amount of A and C vitamins. Its active ingredient that describes the sharp taste is known as capsaicin which has high impact on reduction of body fat mass and cholesterol levels, it was reported that (Song et al. 2014). Red Pepper production has risen 21% since 1994 and plays an important role in all kinds of food, dressings and seasonings, it was reported that (Neetoo & Chen 2012). Red Pepper contains large amounts of carotenoids and ascorbic acid which has high anticancer effects, it was reported that (CRC handbook of medicinal spices.). Before applying any decontamination method, this useful material contains lots of microbial loads along with huge amounts of mold, yeast, and big varieties of bacterial loads, it has been known that (Kim et al. 2014; Rico et al. 2010), and is considered as a contaminator and even infectious substance in food materials, it was reported

that (Neetoo & Chen 2012). It specifically leads to microbial spoilage such as Salmonella and E. Coli's activities in uncooked materials, thus this contamination needs to be reduced to its lowest amount available, it was reported that (Rico et al. 2010).

In Mexico's market, 10 out of 27 samples of red pepper are infected by Salmonella. Furthermore, US witnessed one of the greatest epidemic spreads of Salmonella caused by raw red pepper usage. Most such pathogenic substances have their least activity in dry situations. On the contrast they would have their maximum activity in humid and watery circumstances which leads to endangering consumers' health, it has been known that (Neetoo & Chen 2012; Song et al. 2014). Nowadays, legal and financial acts are concentrated on either developing and enhancing microbes de-off techniques based on preservation quality, consumers' health, and safety. Common preservative techniques are as follow:

A. Utilizing Ethylene Oxide fumigation, which is being used for a long time but banned in many countries since their cancer-causing effects, it has been known that

(Kim et al. 2014; Song et al. 2014). The reason lies underneath the accelerated combination of Ethylene Oxide and Chloride or Bromide in order to generate stable mutagenic cancer-causing compositions (i.e. 2-ChloroEthanol and 2-BromoEthanol), it was reported that (Taylor et al. 2014).

B. Using UV lamps in production and packing lines which its decontamination effect is not that efficient which itself is the consequence of UV's low penetration rate, it was reported that (Kim et al. 2014).

C. Using Ozone would be considered as a safe solution because of its appropriate anti-bacterial effects along with having no garbage and toxin as well, and being decomposed completely in face of Oxygen. This will lead to termination of Microorganisms by means of oxidation of cellular components. However, its usage is not efficient in fatty substances since its oxidative rate is measured to be more than standard thresholds, it was reported that (Oliveira et al. 2013).

D. Utilizing Gamma irradiation, from 2 up to 7 KGy has efficient and significant effect on decontamination of all kinds of spices. Although, other factors such as public fear and high costs, leads to cold reception in many countries, it has been known that (Song et al. 2014; Kim et al. 2014).

E. using heat treatment supported by saturated steam is an efficient approach which performs sterilization properly. However, it leads to unwanted organoleptic and nutritional effects in the specified spice. Moreover, there is a mechanism needed for drying the wet pepper, it was reported that (Kim et al. 2014).

F. Microwave treatment, Microwave's heat has lots of application among food industry, which in controlled situations can act as a beneficial approach in order to reduce microbial load, leading to high performance and qualified product compared with other common heat treatments, it has been known that (Puligundla et al. 2013; Hamoud-Agha et al. 2014).

Losing the quality, including color, odor, texture and taste, is related to remained plant's enzymatic activity such as Polyphenol oxidase, Peroxidase and Lipoxygenase. This negative impact emerges boldly in

their utilization of other food products and leads to product's quality loss. Hence, this enzymatic activity needs to be ceased through heat treatment and not through other non-thermal approaches including fumigation with Ethylene Oxide gas, Gamma irradiation, UV radiation and etc., it was reported that (Schweiggert et al. 2005). Preservation techniques is designed in a way that, with reduction in Pathogen vegetative cells and Spore form, it would be possible to reach to a qualified healthy and safety level. Most of these preservative techniques are developed for highly moisturized products. However dry substances have lots of application in food industry. So, for increasing microbial quality concentrating on dry materials such as spices must be applied. On the other hands, decontaminating dry materials is really troublesome due to adjustment of existed microflora with low moisture content. Heat resistant in micro-organisms depends on water activity (aw) and atmosphere's relative humidity equilibrium. Water existence is the most effective factor in microbial heat resistance. Since, heat resistance in dry environments is existed in much greater amounts rather than in Aqueous Solutions, it was reported by (Fine & Gervais 2004).

II. METHODS AND MATERIAL

A. Samples Preparation

Initially, 3 samples had been made out of a 110 KG pepper's batch, each 300 grams, then mixed thoroughly and randomly divided it into 3 parts again. Each part was used in 3 approaches for a mixture of steam and microwave process, one for Gamma irradiation up to 5 KGy and the last one for control.

1) Sample preparation for the steam-microwave process Fig. 1:

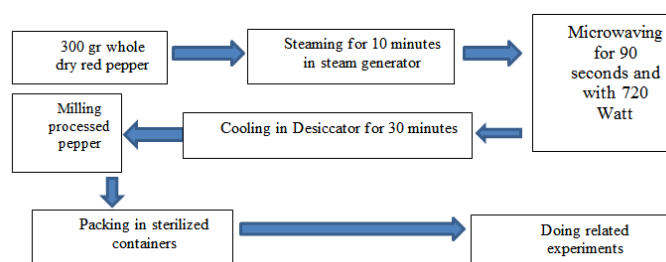


Figure 1: Sample preparation for the steam-microwave process

It can be concluded that, this process has two stages:

- Steaming with a Feller (from Germany) steam generator machine, in order to condition, reduce microbial load and cease enzymatic activities.
- Microwaving with a MC-3022 snr machine produced by LG, in order to dry the conditioned red pepper, continue both microbial load reduction and cease enzymatic activities.

2) Sample preparation for Pepper Irradiating process

Following milling the second batch and putting them in sterilized containers, they are sent for Gamma irradiation up to 5 KGy for about 34 min and 12 second. Source host configurations of Irradiation device are as follows:

- Dos Rate: 2.56 Gy/Sec
- Transit Rate: 7.86 Gy

After irradiating the second batch, they are sent to laboratory for microbial and chemical experiments.

3) Sample preparation for control

Control sample is the one with no applied processes and is just milled and packed with means of comparing with two other samples.

B. Microbial experiments

1) Total Plate Count Agar based on Iran's National Standard (5272):

The counting process for specified colonies is performed by a standard procedure which includes adding prepared 0.1 dilution of Pepper suspension to the culture medium of PCA (Merck, Germany) which has a rotational movement, then flocculating the specified culture and finally transporting it into Incubator in an upside down position for 72 hours in 30°C.

Counts the number of colonies with following formula:

$$N_E = Y/d$$

d: coefficient of initial dilution suspension.

Y: Average number of existed colonies in two plates.

2) Counting Mold and Yeast below 0.6 water activity based on Iran's National Standard (10899-3):

This experiment is performed in a Yeast extract-Dextrose-Chloramphenicol (YGC) culture medium. The counting process for specified colonies is performed by a standard procedure which includes adding prepared 0.1 dilution of Pepper suspension to the culture medium of YGC (Merck, Germany) which has a rotational movement, then flocculating the specified culture and finally transporting it into Incubator in an upside down position for 5 days in 25°C.

Counts the number of colonies with following formula:

$$N_E = Y/d$$

d: coefficient of initial dilution suspension.

Y: Average number of existed colonies in two plates.

3) Counting Coliform based on Iran's National Standard (9263):

Its culture medium is Crystal violet, neutral red and bile lactose agar (VRBL). The counting process for specified colonies is performed by a standard procedure which includes adding prepared 0.1 dilution of Pepper suspension to the culture medium of VRBL (Merck, Germany) which has a rotational movement, then flocculating the specified culture, supported by adding 4 ml from VRBL to each plate, and finally transporting it into Incubator in an upside down position for 24 hours in 30°C.

4) Counting E.coli based on Iran's National Standard (2946):

This process is performed by adding 1 ml of initial suspension to 9 ml of Lauryl sulphate broth. The Inoculated tubes are held in incubator in 37°C in periods of 24 and 48 hours. In case of gas production in Durham tubes, EC and all supplementary tests are held, otherwise the report is sent as negative.

C. Physicochemical experiments

1) pH measurement based on Iran's National Standard (2852):

10 grams of red Pepper is mixed with 50 ml of distilled water, followed upon measuring its pH by a pH meter

electrode (Jenway, Germany) and after fixing its number, it will be reported.

- 2) Moisture measurement based on Iran's National Standard (2705) for calculating Total Solid (TS):

After weighing 5 grams of the sample (M2) and the container with its lid (md), it will be put in oven (Memert, Germany) for 120 minutes, followed by cooling in desiccating machine for 30 minutes. Then, it will be weighed (ml) upon which the dried sample mass (M1) is extracted by below formula:

$$M1=ml-md$$

In order to calculate moisture percentage following formula can be used:

$$W_{H_2O} = \left[1 - \frac{m_1}{m_2} \right] * 100$$

- 3) Qualitative determination of enzymatic activity (Catalase and peroxidase) based on Iran's National Standard (48):

On the cross section of a gram of different treatments, a drop of 3.0 or 5.0 per cent hydrogen peroxide solution (Merck, Germany) is added. With emergence of pink colour tinged with orange, or creation of lots of bubbles, the existence of Catalase and peroxidase enzymes is assured in the specific sample.

- 4) Peroxide test based on Iran's National Standard (4179):

First oil in pepper is extracted with cold extraction, and then 5 grams of the oil is mixed with 50 ml of solution (acetic acid (Sigma Aldrich, US) + iso-octane (Merck, Germany) which is prepared in a 3 to 1 proportion. Then, 0.5 ml of saturated potassium iodide (KI) (Merck, Germany) is added to the container. Then the container is left in a dark cabinet for 1 minute. Following stage, 100 ml of distilled water is added to the container with a few drops of starch (Kimia Gostar, Iran) (1 to 5 percentage starch solution).

A prepared sample which is now in dark colour, will be under the affection of titration with 0.01 N sodium thiosulfate (Sigma Aldrich, US) up until it becomes colourless. Besides, Titration of the control is performed.

Thiosulfate amount consumed by the control is subtracted from the amount consumed by the sample.

Amount of proxide:

$$\frac{N * (S - B) * 1000}{W}$$

Hereby the parameters are as followed:

S: Thiosulfate volume consumed by the sample

B: Thiosulfate volume consumed by the control

N: Thiosulfate Normality

W: Sample Weight

D. Machinery Tests

- 1) Colorimetric measurement with Hunter lab system
a*, b* and L* colour space is a model which was approved in 1976 by International Commission on Illumination light for describing the relationship between the visible colours by eye in which L* defines brightness (between 0 to 100), a* represents red and greenish colour (+a for red and -a for green) and b* explains yellow and blue colours (+b for yellow and -b for blue), that both a* and b* are from -120 to +120.

E. Statistical Analysis

By using Microsoft Excel in representing and packing the raw data and utilizing one-sample T-test from SPSS software the data was summarized, interpreted and observed properly.

III. RESULT AND DISCUSSION

A. Total Solid results with $P < 0.01$ assumption

According to Fig. 2, Steam-microwave process (B), had the lowest TS compared to the control (C) and irradiated sample (A), which B's TS is less than about 0.64 of control sample averagely, Rico et al.(Rico et al. 2010) have shown that.

Employing heat steam-microwave process leads to increase in Moisture content and the reason lies underneath the facts of water absorption during steaming process and Failure in returning the moisture to its initial amount during Microwave process. In spite of the fact that these changes are considered as really minute (about 0.6%) and would have no effects on pepper's preservative and organoleptic properties.

In irradiated sample, TS rose to a small extent and this is due to the fact that there were some structural failures in molecular combinations, subsequently some moisture loss during Irradiation.

B. pH results with $P < 0.01$ assumption

According to Fig. 2, pH alterations after both A and B processes had negligible differences to control sample, have been reported (Rico et al. 2010; Lu et al. 2011; Valero & Cejudo 2014) and there was only 0.2 increases in pH value by utilizing Gamma irradiation in average.

pH alterations for both two processes compared to control sample were minimal. In other hand, Gamma Irradiation had a slice increase for 0.2 which might be because of broke Amine compounds followed by pH increase.

C. Peroxide index (meq/Kg o2) results with $P < 0.01$ assumption

It was observed in Fig. 2, that Peroxide index in all samples was tightly close to each other, however there was an insignificant increase in Peroxide amount for Irradiation sample (A), Aziz et al. (Aziz et al. 2002) reported that and those which were used in steam-microwave process had the least Peroxide amount among all other.

Peroxide index in both A and B approaches were highly similar to each other and even to control sample (C), but there was some negligible decrease in irradiation technique which was due to the fact that oil content oxidation is happened in irradiation.

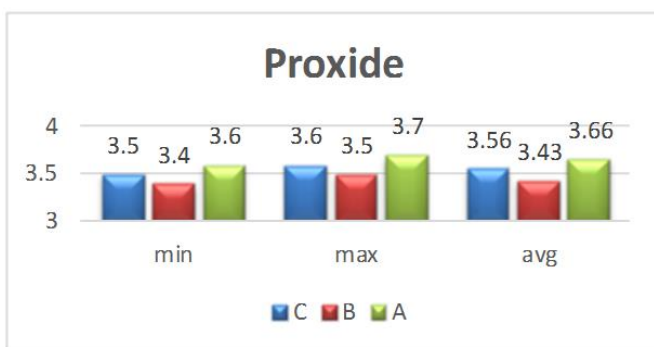
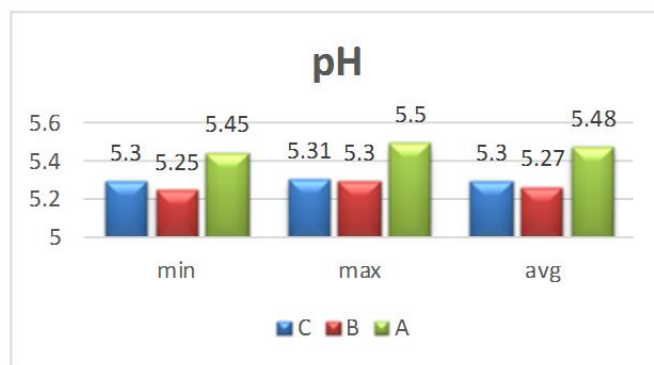
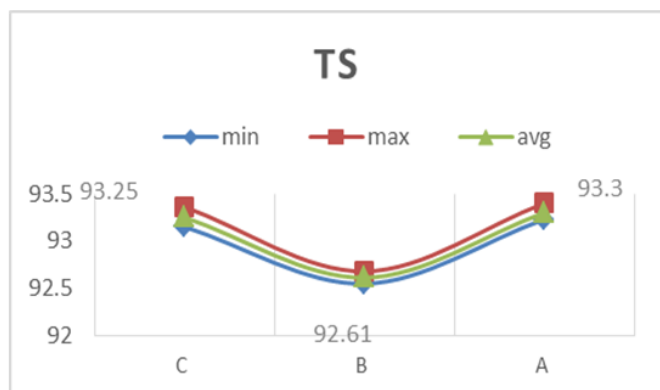


Figure 2 : TS, pH, and peroxide amount curve for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample)

D. Coliform results with $P < 0.01$ assumption

According to Fig. 3, Coliform amount, in the control sample (C) is about 256 averagely, while in Steam-microwave process (B) and Irradiated sample (A), this factor was reached to zero, have been reported (Kim et al. 2014; Aziz et al. 2002; Schweiggert et al. 2005).

In both steam-microwave (B) and Gamma Irradiation (A) approaches, Coliform had gained well-received desirable zero amounts which are conceived as their high effective intensity in Coliform termination.

A. E.coli results with $P < 0.01$ assumption

As it is observed in Fig. 3, E.coli amount was about zero in control sample, consequently in processed samples (A and B) there would not be any E.coli bacteria, it has been known that (Hamoud-Agha et al. 2014; Song et al. 2014) result.

There was no evidence on E.coli existence in the control sample (C), and consequently there were same results for other two approaches (A and B).

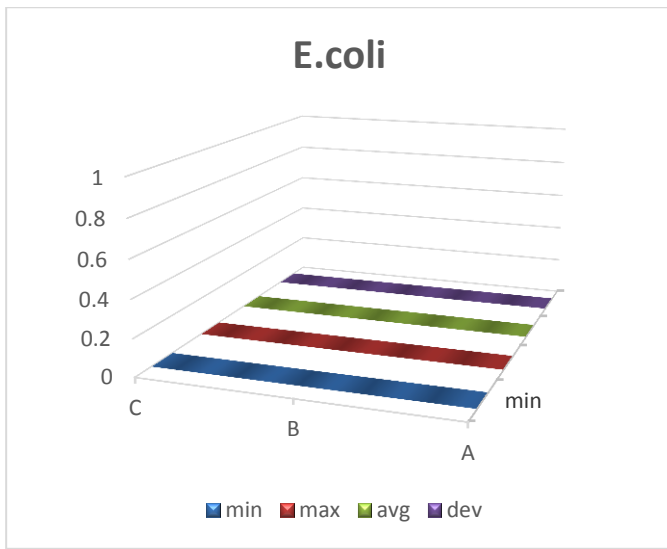
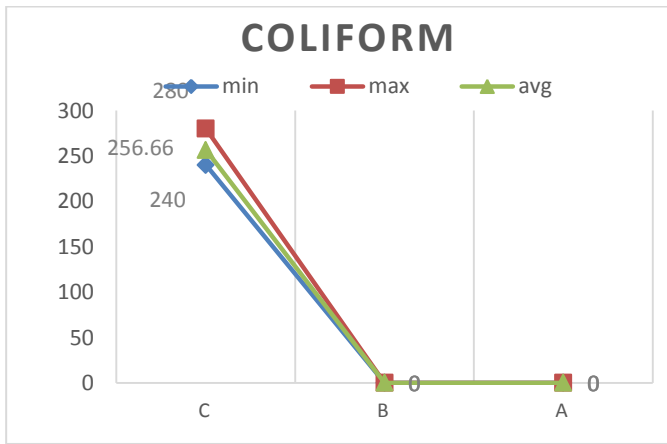


Figure 3 : Coliform and E.coli amount curve for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample).

B. Total Plate Count Agar (TPCA) results with $P < 0.01$ assumption

According to Fig. 4, There was high amounts of TPC in our initial sample (C), nearly about 1287, but with employing the steam-microwave process (B) for 3 times, this number is reduced to about 56 colony, have been reported (Rico et al. 2010; Aziz et al. 2002; Schweiggert et al. 2005) .

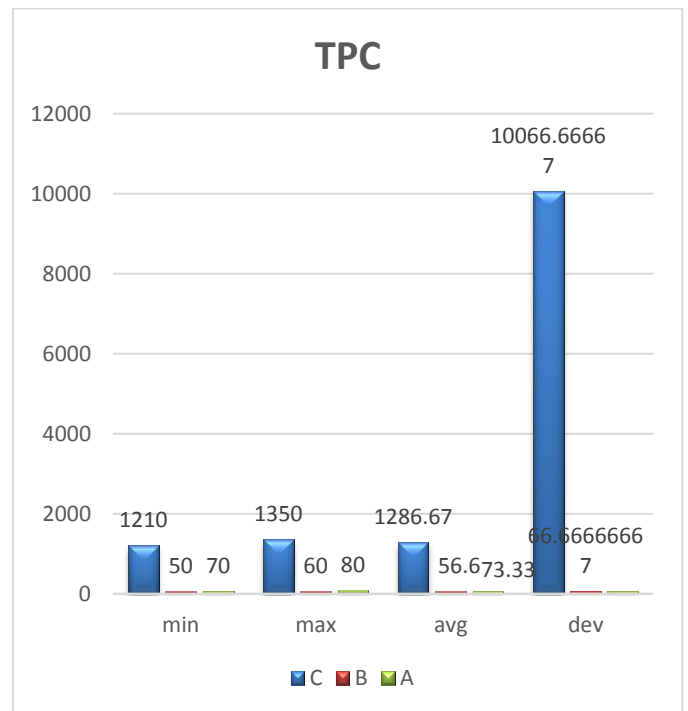
Besides, by applying irradiation this number is reduced to about 73 colonies, it has been known that (Aziz et al. 2002; Rico et al. 2010).

The most significant reduction of TPC was seen in steam-microwave process which on average decreased the number from 1286 in control sample (C) to 56, which is followed by next technique in second position, Gamma Irradiation that lessened the number to 73 units of TPC.

C. Mold results with $P < 0.01$ assumption

According to Fig. 4, Initial Mold count on our control sample (C) was about 3733, but with employing the steam-microwave process (B) or applying Gamma irradiation with 5 KGy, there was no other signs of mold observed in culture media, it has been known that (Schweiggert et al. 2005; Rico et al. 2010) .

Mold values in control sample was high in amounts initially, for about 3737, but with applying steam-microwave (B) and Gamma Irradiation (A) upon processing its amount declines drastically to the state of no mold in both samples which explains highly responsive behaviour of mold destruction in both processes.



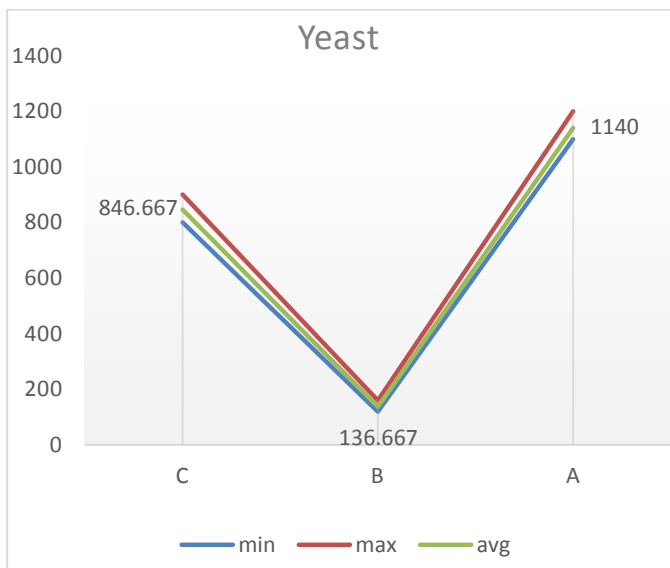
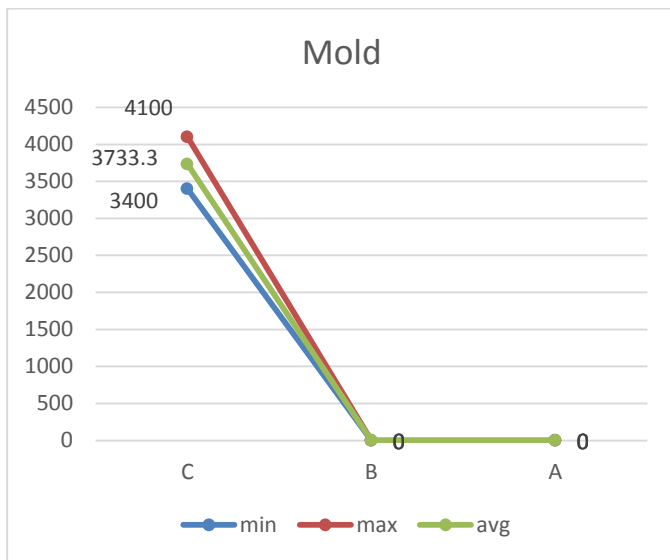


Figure 4 : TPC, Mold, and yeast amount curve for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample).

D. Yeast results with $P < 0.01$ assumption

According to Fig. 4, There was high amounts of Yeast in our initial red pepper sample (C), nearly about 846, however by employing the steam-microwave process (B), this number is reduced to about 136, it has been known that (Schweiggert et al. 2005; Rico et al. 2010). Besides, by applying irradiation with 5 KGy in 3 times, this number is increased to about 1140, in contrast with the data, Rico et al. (Rico et al. 2010) have shown that, due to lower treatment dose irradiation.

The lowest yeast amount is referred to steam-microwave process (B) in which the amount was reduced from 846 of control sample (C) to 136 units. However, employing Gamma Irradiation approach (A), led to its soar to 1140 which was due to the fact that Irradiation causes germination of yeast cells. This malfunction creates disastrous problems which itself is the key factor in spoilage and fluff in future products.

E. Hunter Lab Colorimetric results

1) L^* (Brightness factor) with $P < 0.01$ assumption

According to Fig. 5, In steam-microwave approach L^* amount was about 44 which was reduced from the control sample value by 3 units, have been reported (Schweiggert et al. 2005; Rico et al. 2010; Chang et al. 2010) result, although there was a 3 unit increase on this factor by employing Gamma Irradiation (A) approach compared to control sample (C), Song et al. (Song et al. 2014) have shown that.

2) a^* (Red factor for red pepper) with $P < 0.01$ assumption

According to Fig. 5, On average, a^* index was about 22.11 in the steam-microwave approach (B), which had a reduction compared to control sample (C) with its amount near about 23.7, have been reported (Schweiggert et al. 2005; Rico et al. 2010; Chang et al. 2010) result, although in Gamma Irradiation approach (A) this index was increased in small amounts to 24.61, Song et al. (Song et al. 2014) have shown that.

3) b^* (Yellow factor for red pepper) with $P < 0.01$ assumption

According to Fig. 5, b^* index had a decrease in the steam-microwave approach (B), and its amount declined from 51.6 for Control sample (C) to 49.4, have been reported (Schweiggert et al. 2005; Rico et al. 2010; Chang et al. 2010) result. Although in Gamma Irradiation approach (A) this index was increased for about 5 units which lead to more yellow color in its output, Song et al. (Song et al. 2014) have shown that.

All in all, steam-microwave approach had tiny effect on reduction of brightness (L^*), red color (a^*) and yellow

color (b^*) factors, which is due to the fact that there are some destructions in colour pigments during steaming and microwaving processes. In other hand, irradiation led to minuscule upward trend in all color factors that are the result of destruction of some pigments during Gamma irradiation along with de-polymerization of substances and structural unpacking.

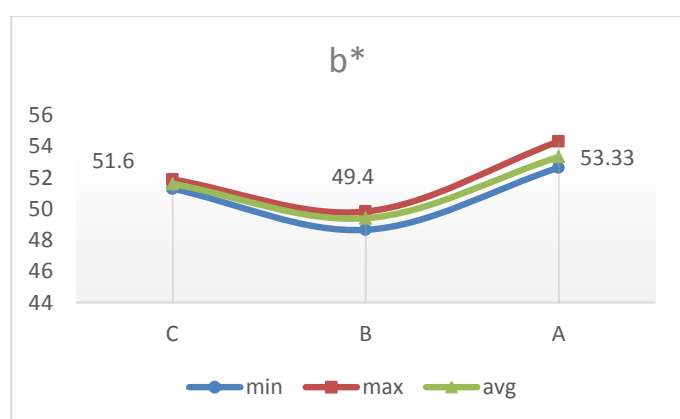
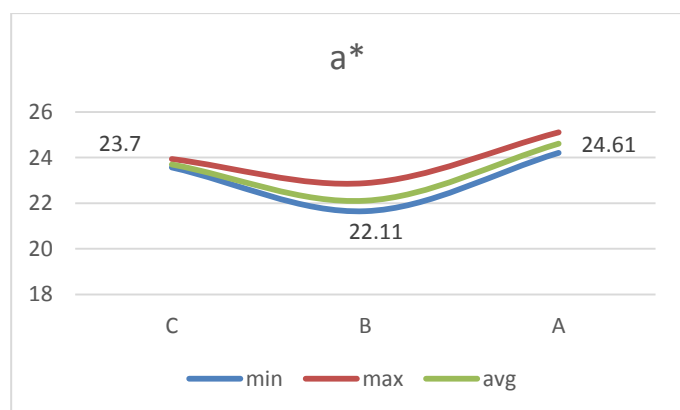
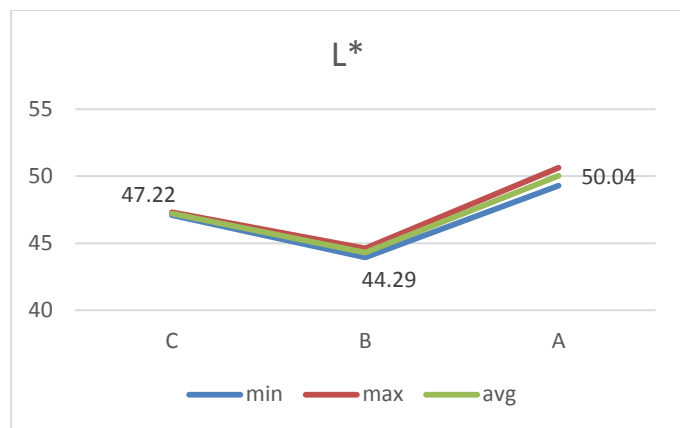


Figure 5: L^* , a^* , and b^* amount for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample)

F. Existence Quality Test of Enzymatic Activity

According to the table 1, both control and Gamma irradiated samples had enzymatic activities which led to negative effects on its future products; however the output from steam-microwave process lacked any enzymatic activities. Schweiggert et al. (Schweiggert et al. 2005) has shown that.

Table 1: Existence Quality Test of Enzymatic Activity.

Condition	Control	Steam-Microwave	Gamma Irradiated
Enzymatic Activity			
No Enzymatic Activity			

IV. CONCLUSION

Steam-microwave process (B), had the lowest TS compared to the control (C) and irradiated sample (A), pH alterations after both A and B processes had negligible differences to control sample, Peroxide index in all samples was tightly close to each other; however there was an insignificant increase in Peroxide amount for Irradiation sample (A), Coliform amount, in the control sample (C) is about 256 averagely, while in Steam-microwave process (B) and Irradiated sample (A), this factor was reached to zero, E.coli amount was about zero in control sample, consequently in processed samples (A and B) there would not be any E.coli, The most significant reduction of TPC was seen in steam-microwave process, The most significant reduction of mold was seen in steam-microwave process, The lowest yeast amount is referred to steam-microwave process (B) However, employing Gamma Irradiation approach (A), led to its soar, All in all, steam-microwave approach had tiny effect on reduction of (L (a^*) and (b^*) factors,. In other hand, irradiation led to minuscule upward trend in all colour factors and both control and Gamma irradiated samples had enzymatic activities which led to negative effects on its future products; however the output from steam-microwave process lacked any enzymatic activities.

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