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Influence of Gamma Radiation on Microbial and Viscosity Parameters of Henna Based Hair Colour

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Abstract— **Henna has been used as a cosmetic hair dye for 6,000 years. The leaves are reduced to fine powder and mixed with water, to form a paste, which is applied to the hair for deliver the colour and conditioning to the hair. In recent years, the dye intermediate and oxidizing agent like sodium perborate/ barium peroxide also mixed with henna powder to deliver the desired colour to the hair. The combinations of selective dyes, oxidation agent, along with henna powder deliver the variety of shades to the hair. Article deals with the influence of gamma radiation among the colour delivery, microbial population and viscosity of henna paste. Exposure the henna powder in to gamma radiation does not affect the colour delivery; gamma radiation does not affect the dye intermediates as well as lawsone content also. Gamma irradiation of henna powder drastically reduced the microbial population when compared to the untreated control. Accidently noticed that the viscosity of henna paste dropped by gamma irradiation process. Exclusively articles deals with the optimum dosage of gamma radiation with respect to viscosity and microbial population. Study results revealed that optimum dosage is 8 KGy in terms of microbial and viscosity parameters. Irradiation of henna powder has to improve the hygienic quality of the product and extended the shelf-life of the products and it is stand alone in the export markets/international business.**

Index Terms — **Henna Hair Colour, Gamma Irradiation, Sodium Carboxy , Methyl Cellulose, Microbial Population.**

I. INTRODUCTION

Henna has been used as a cosmetic hair dye for 6,000 years. Henna has also traditionally been used for centuries in other parts of North Africa, the Horn of Africa, the Arabian Peninsula, the Near East and South Asia. The henna plant (Lawsonia inermis L.) contains a red-orange component, 2-hydroxy-1,4-napthoquninone is the active constituent in henna leaves and which is important for deliver the colour. Henna has been used as a hair dye since the dawn of history and is still commonly employed as a conditioner and coloring agent in cosmetic preparations for the hair [1] [2]. The leaves are reduced/grained to fine powder and mixed with water, to form a paste, which is applied to the hair for deliver the colour and conditioning to the hair. However it will deliver the reddish orange shade to the hair. In recent years, the dye intermediate like para phenylene diamine and its derivative and oxidizing agent like sodium perborate/ barium peroxide also mixed with henna powder to deliver the desired colour to the hair. The combinations of dye and oxidation agent along with henna powder deliver the variety of shades to the hair. In present study composition consisting the dye material, oxidizing agent, buffering agent, thickener, antioxidant, filler and henna powder. Dye intermediate and oxidizing agent used for deliver the desired colour delivery, in general citric acid and tartaric acid used as a buffering agent and it use for maintaining the pH range between 6 and 8.

Sodium carboxyl methyl cellulose, xanthangum, hydroxyl ethyl cellulose are act as a thickener. In general the products are sold in 10 g pouch and recommended to dilute 35 to 40 mL water for make a paste and colour delivery /developmental time is 30 minutes. Thickener in henna hair colour formulations are immense useful to make the paste consistency and avoid the dripping during the application time. Sodium sulphite, ascorbic acid, butyl hydroxyl toluene, etc. can be used as an antioxidant and it prevents the oxidation of dye intermediate during self life time. Approximately around 30 to 60 % henna powder added in the composition to give nourishment and shine to the hair. However henna powder is a commodity, it is cultivated and harvested in various seasons. It containing high amount of microbial population, according to the regulatory aspects [3], the consumer products are having less than 100 cf total bacterial count and less than 10 cfu/gm total yeast and mould count. In general micro containing products treated with gamma radiation and reduces the microbial population to desired level as per the regulatory norms. Literature study revealed that higher dosage of gamma radiation affects the thickness/viscosity of the final product. Various literatures are available in terms of influence of gamma radiation on microbial property and sodium carboxy methyl cellulose [6] to [10]. However there is no authentic document are available on henna hair colour with respect to gamma radiation. We have aimed to develop the document on influence of gamma radiation on henna hair colour. This article deals about the optimum dosage of gamma radiation of henna hair colour product, exclusively studied the dosage of gamma radiation on microbial population and also studied the influence of gamma radiation on viscosity of the henna paste.

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I. MATERIALS AND METHODS

A. Preparation of Herbal Henna Hair Colour

Article exclusively deals with the two shades of henna based hair colour natural black shade and dark brown shade respectively. Skeleton formulation are furnished in the below table 1. Initially first heat the henna powder to 80 to 85 °C and remove the moisture content then add the buffering agent, filler and anti oxidant and mix for 5 to 10 minutes. Further add the dye intermediates and mix for 10 minutes. Finally add the oxidizing agent and mix for 30 minutes.

Table 1. Skeleton formula of henna hair colour

S.No.	Materials	Natural black shade	Dark brown shade
		%	%
1	Henna powder	Q.S	Q.S
2	Dye intermediates	10	8
3	Fillers	20	20
4	Oxidizing agents	15	15
5	Buffering agents	5	5
6	Anti oxidants	0.5	0.5

Table 2: **Influence of gamma irradiation on chemical parameters of hair colour powder**

Shades			Natural black		Dark Brown	
S.No.	**Test**	**Specification**	**Control (without gamma treatment)**	**After treatment**	**Control (without gamma treatment)**	**After treatment**
1.	Description	Greenish to Grayish Color Powder, having characteristics odor	Greenish powder	Greenish powder	Greenish powder	Greenish powder
2.	pH of 5% (m/m) solution with water at 27±2ºC	6.00 – 9.00	7.72	7.77	8.56	8.45
3.	Available of Oxygen w/v (%)	NLT 0.70	0.82	0.87	0.85	0.82
4.	Para Phenyline Diamine Content % by mass	NLT 7.00 for black NLT 3.0 for brown	8.65	8.72	4.56	4.42

Table 3: Influence of gamma radiation on microbial population of herbal henna hair colour.

	Shades	**Natural Black**		**Dark brown**	
		Total Aerobic Bacterial count (CFU/g)	**Total Yeast & Mould *(CFU/g)***	***Total Aerobic Bacterial count (CFU/g)***	**Total Yeast & Mould *(CFU/g)***
1	Control – without gamma radiation	1488	<10	924	<10
2	Gamma radiation @ 5KGy	148	<10	<10	<10
3	Gamma radiation @ 8KGy	<10	<10	<10	<10
4	Gamma radiation @10KGy	<10	<10	<10	<10
5	Gamma radiation @12KGy	<10	<10	<10	<10
6	Gamma radiation @20KGy	<10	<10	<10	<10

Viscosity measurements:
Viscosity of henna hair colour sample measured with Brookfield viscometer (Model - RVDV 1+; 2014). 10 gm of powder dissolved in 40 mL water and after complete mixing making the paste and measure the viscosity at various time intervals like after 15 minutes, 30 minutes etc.

Table 4: Influence of gamma radiation on viscosity of herbal henna hair colour.

	Shades	**Natural Black**		**Dark brown**	
		15 minutes	**30 minutes**	**15 minutes**	**30 minutes**
		Viscosity# CPS (10 g henna hair colour + 40 mL of water)			
1	Control – without gamma radiation	1250	1300	1400	1450
2	Gamma radiation @ 5 KGy	873	890	1000	1050
3	Gamma radiation @ 8 KGy	574	590	750	770
4	Gamma radiation @ 10 KGy	350	351	400	415
5	Gamma radiation @ 12 KGy	320	323	360	365
6	Gamma radiation @ 20 KGy	250	259	260	268

#RV-5 spindle; 25 rpm

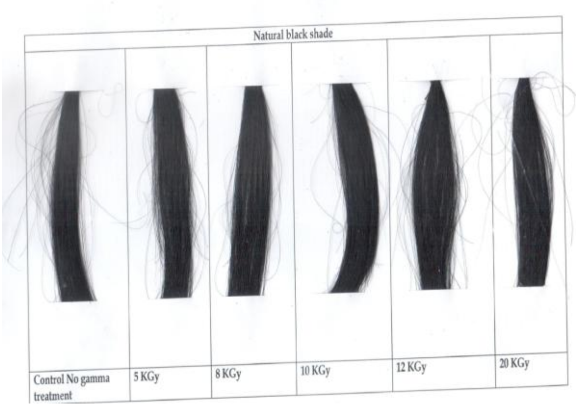

Fig 1: Influence of gamma radiation on colour delivery – Natural black shade

Fig 2: Influence of gamma radiation on colour delivery – Dark brown shade

A. Micro estimation:

Presence of micro-organisms was detected as per GSO guidelines [3]

Using Petri dishes 9 cm in diameter, add to the Dish 1 ml of sample prepared and 15 ml to 20 ml Casein soya bean digest Agar or Sabouraud –dextrose agar, both media being at not more than 45°C. If larger Petri dishes are used, the volume of the agar is increased accordingly. Spread a measured volume of not less than 0.1 ml (1 ml is appropriate) of the sample prepared as described in the section Preparation of the sample over the surface of the medium. Dry the plates, for example in a LAF bench or in an incubator Use at least two Petri dishes for each medium and each level of dilution. Allow the media plate to solidify. Incubate the Soyabean casein digest. Agar plates at 30-35oC for 3 to 5 days and Sabouraud Dextrose agar Plate at 20-25oC for 5 -7 days. Take the arithmetic mean of the count per medium and calculate the number of CFU in the original inoculums.

Using Petri dishes 9 cm in diameter add 15 ml to 20 ml Casein soya bean digest agar or Sabouraud –dextrose agar at about 45°C to each Petri dish and allow to Solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a LAF bench or in an incubator. Spread a measured volume of not less than 0.1 ml (1 ml is appropriate) of the sample prepared as described in the section Preparation of the sample over the surface of the medium. Use at least two Petri dishes for each medium and each level of dilution. Allow the media plate to solidify. Incubate the Soyabean casein digest Agar plates at 30-35oC for 3 to 5 days and Sabouraud Dextrose agar Plate at 20-25oC for 5 -7 days. The contents were mixed and the plate was allowed to set. This was followed by incubation at 23°C for 5 days and daily examination for the count. Take the arithmetic average of the count and calculate the number of colony forming units per gm or ml of the Product

B. Gamma radiation:

Gamma sterilization is performed after packaging of the products in the final containers / packs and does not involve any septic handling. Various dosages of gamma sterilization like 0 KGy (control without sterilization), 5KGy, 8KGy, 10KGy, 12KGy and 20KGy at Shriram Institute of Industrial Research, Delhi, India.

C. Swatch assay:

Colour delivery was estimated in hair swatches. 10 g of henna hair colour mixed with 40 mL of distilled water and mix for 5 minutes. After 5 minutes henna paste mixture applied to the hair swatches and wait for 30 minutes developmental time. After 30 minutes the hair swatches was washed with water and dried in air. Swatch assay is immense useful to identify the colour delivery and shade variation/shade direction.

II. RESULTS & DISCUSSION

A. Influence of gamma radiation on colour delivery:

Colour delivery of gamma treated henna hair colour compared with untreated henna hair colour. Swatch assay reveled that gamma radiation does not affect the colour delivery with respect to higher dosage also. Swatch results are furnished in Fig. 1 &2.

Untreated control sample and higher dosage of gamma irradiation sample deliver the black shade in hair swatches with respect to natural black shade. Similar kind of trend was noticed in brown shade also. Application of gamma radiation does not affect the colour delivery; it confirms that the dye intermediate and oxidizing agents are stable even after pass-through the gamma radiation. After gamma irradiation does not affect the dye intermediate concentration in the formula and overall analytical data combined in Table 2. Results revealed that there is no significant difference in chemical parameters due to the gamma treatment.

B. Influence of gamma radiation on Microbial counts:

Various dosage of gamma radiation passed through the herbal henna hair colour formula and the results are summarized in the table 3. Results revealed that untreated henna hair colour (i.e without gamma treated) shows the 1488 cfu/g of total aerobic bacterial count and 148 cfu/g of total aerobic bacterial count was noticed in 5KGy gamma irridated sample of Natual black shade. However in dark brown shade 924 CFU/g total bacterial counts noticed in untreated powder and less than 10 CFU/g aerobic bacterial count noticed in 5 KGy onwards. With 8KGy gamma irradiation sample onwards the microbial population falls under less than 10 CFU/g. Study results clearly indicate that the optimum dosage level of gamma radiation is 8KGy for henna based hair colors. The decontaminating the products using gamma radiation helped in meeting the required standard. Irradiation is one of the few processes that allow the attainment of high standards for foods and medical products through the destruction of bacteria and other micro organisms. Gamma irritated sample shows the acceptable limit of microbial population / counts, the main reason behind that, by which radiation kills micro organisms is by splitting water molecules into hydrogen (H^+), hydroxyl (OH^-) and oxygen (O-2)radicals [9] [11]. Irradiation has the capacity to improve the hygienic quality product and extended the shelf-life with quality. It give the competitiveness an especially for the export markets/international business to the herbal based products.

C. Influence of gamma radiation on viscosity:

40 mL of water added in 10 gm of henna powder and mix for 2 to 3 minutes for uniform mixing for swelling the thickener. The same sample was measure the viscosity using viscometer and results are furnished in Table. 4. Untreated samples paste shows the 1250 cps viscosity, however the higher (20 KGy) dosage of gamma irradiated sample shows 250 CPS viscosity with respect to the black shade; similar kind of trend was noticed in brown shade also. We measure the viscosity after 15 minutes mixing interval and 30 minutes mixing interval. There is no significant difference between 15 minutes and 30 minutes. Application of higher amount of gamma radiation drastically reduced the viscosity of the henna hair colour powder, in which our formula sodium carboxy methyl cellulose acts as a thickener. It helps to increase the viscosity and consistency of paste. Carboxymethylcellulose is a cellulose derivative with carboxymethyl groups bound to the hydroxyl groups of the glucose unit, and it is an industrially important cellulose derivative, primarily due to its high viscosity, non-toxicity and non-allergic character [12]. During irradiation with gamma radiation the polymer structures are broke down and form the individual glucose unit [13] [14] [15]. Main reason is during irradiation free radical ions are produced and it prevents the cross linking formation and ionization also. Ionization and cross linking structure is very important for swelling the sodium carboxy methyl cellulose to form a desired viscosity. In addition to that the free radicals breakdown the cellulose unit into small fragments and prevent the ionization.

III. Conclusion

Application of gamma radiation does not affect the colour delivery; it confirms that the dye intermediate and oxidizing agent are stable even after pass-through the higher amount of gamma radiation. Study report clearly indicate that the application of gamma radiation reduce the microbial population to accepted level. Optimum dosage of microbial population is 8 KGy gamma radiations. Study also confirm the application of higher dosage of gamma radiation affects the viscosity of henna paste during the application time, it is due to the cross-linking polymer chain broken due to the gamma radiation. The optimum dosage of gamma radiation is 8 KGy with respect to microbial population and viscosity parameters.

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