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ESTIMATION OF MICROBIAL GROWTH IN THE PROCESSED ORIENS® WHEATGRASS CAPSULE

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Wheat Grass refers to the young grass of the common wheat plant, Triticum aestivum that is freshly juiced or dried into powder for animal and human consumption. Both provide chlorophyll, amino acids, minerals, vitamins, and enzymes. Wheat grass is a humble weed that is a powerhouse of nutrients and vitamins for the human body. Diagnosis of microbial quality of these products is important. In this study, the microbial growth of the processed Oriens® Wheatgrass were done. The microbial tests such as total aerobic bacterial count, mold and yeast count, *E. coli*, *Salmonella sp*. and *Staphylococcus aureus*, were evaluated based on the national standards. Oriens® Wheatgrass was not contaminated with microorganism. The data indicated that Oriens® Wheatgrass had proved to indicate the better quality for consumer health.

Keywords: Wheatgrass, Microbial analysis, Standards, Quality

INTRODUCTION

Functional food components are potentially beneficial components found naturally in foods or added to them which may include carotenoids, dietary fibre, fatty acids, flavonoids, isothiocyanates, phenolic acids, plant stanols, sterols, polyols, prebiotics, probiotics, phytoestrogens, soy protein, vitamins and minerals (Guine *et al.*, 2010). There are many health benefits that are linked to the super foods that are regarded as functional foods. In Eastern cultures these super foods are part of the everyday healthy diet and considered to have therapeutic value. Whereas in some countries, super foods are considered as food supplements by the pharmaceutical and food market (certain foods with documented health benefits to improve health and bring down the health care cost is well known as super foods). It decreases the risk of heart disease, triglyceride level, cholesterol, free radicals and constipation. It supports digestion, contains antioxidants, improves immunity and alleviates inflammatory conditions.

Wheat grass is a sprout from common wheat, Triticum aestivum, harvested before it flowers. It is an edible food/ functional food that is consumed as fresh juice, dried into powder or made into tablets (Sunil *et al.*, 2006; and Rajagopalan and Shakya, 2014). The grass has been established to be highly nutritious being rich in protein/ amino acids (essential and non-essential amino acids), vitamin C, minerals, enzymes and chlorophyll (Chauhan, 2014). According to Ran *et al.* (2011), the nutritional composition of wheat grass per 3.5 grams has been found to contain 860 mg protein, 18.5 mg chlorophyll, 15 mg calcium, 38 mg lysine, 7.5 mg vitamin C and containing abundance of micronutrients and amino acids (Rana *et al.*, 2011).

Quality assurance should be a corporate goal, and should stem from the uppermost management level to the least staff of the industry. Along the supply chain, food is unavoidably exposed to numerous hazards. Therefore, knowing the risk factors at each phase of the supply chain assists in ensuring that an effective and comprehensive

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quality system is put in place. To guarantee food quality, it is important to ensure that, all the steps of the supply chain are carried out strictly with care and according to the standard operating procedures. At each phase of the supply chain, the potential risks, responsibilities and how they can best be addressed must be fully explored (Edith and Ochubiojo, 2012).

Biological Hazards are caused by bacteria, viruses or parasites present in air, food, water, soil, animals and humans. Food infections and food intoxications can cause severe vomiting, diarrhea, nausea, abdominal pain and fever to one or lots of people, they can even result in death in some serious cases. Biological hazards receive the most attention in Hazard Analysis Critical Control Point (HACCP) systems due to presenting the greatest risk of harm and the highest frequency of occurrence.

Microbial contamination is assumed to occur through handling by personnel who are infected with pathogenic bacteria during harvesting/collection, post-harvest processing and the drug manufacturing process. This should be controlled by implementing best practice guidelines such as Good Manufacturing Practice-GMP (WHO, 2007). The aim of the present study is to determine the microbial quality of Oriens® Wheatgrass capsule to ensure the safety of the product.

METHODOLOGY

Detection of E. coli

Materials and Chemicals Required

Mac Conkey agar or Tregitol agar, Distilled water, Autoclave, Disposable Petri plates, Colony counter.

Methods

Accurately weighs the sample of 1gm and dissolve the 10 ml of distilled water to prepare a test sample. Then prepare a specific agar powder (MacConkey agar or Tregitol agar) weighing the quantity as per guidelines given in the medium bottle). Add weighed agar powdered in conical flask followed by required amount of water and sterilized at autoclave at 15 lbs (121 °C) for 15 minutes.

After sterilization, the agar is kept cooled at cabinet for 10 min to cool, the freshly prepared agar is poured in new petri plates and allowed to form gel, and the freshly prepared test sample is inoculated in the agar. Incubate the petri plates at 30-35 °C for 24 hrs. After 24 hrs, and bacterial of colonies presence or absence is detected and counted using a colony counter.

Detection of S. aureus

Materials and Chemicals Required

Mannitol salt agar or Baird parker agar, Distilled water, Autoclave, Disposable Petri plates, Colony counter.

Methods

Accurately weigh 1 g of the herbal or food sample and dissolve in 10 ml of distilled water to prepare a test sample. Then prepare a specific agar powder (mannitol salt agar or Baird parker agar) weighing the quantity as per guidelines given in the medium bottle. Add weighed agar powder in conical flask followed by required amount of water and sterilize by autoclaving at 15 lbs (121 °C) for 15 minutes. After sterilization, the agar is kept for cooling in the cabinet for 10 min. The freshly prepared agar is poured in new disposable petri plates and allowed to solidify. The freshly prepared test sample is inoculated in the agar. Incubate the petri plates at 30-35 °C for 24 hrs. After 24 hrs presence or absence of bacterial colonies is detected and counted using a colony counter.

Detection of Salmonella

Materials and Chemicals Required

Bismuth brilliant green agar, Distilled water, Autoclave, Disposable Petri plates, Colony counter.

Methods

Accurately weigh 1 g of the herbal or food sample and dissolve in 10 ml of distilled water to prepare a test sample. Then prepare a specific agar powder (Bismuth brilliant green agar) weighing the quantity as per guidelines given in the medium bottle, Add weighed agar powder in conical flask followed by required amount of water and sterilize by autoclaving at 15 lbs (121 °C) for 15 minutes. After sterilization, the agar is kept for cooling in the cabinet for 10 min. The freshly prepared agar is poured in new disposable petri plates and allowed to solidify. The freshly prepared test sample is inoculated in the agar. Incubate the petri plates at 30-35 °C for 24 hrs. After 24 hrs presence or absence of bacterial colonies is detected and counted using a colony counter.

Detection of Total Bacterial Colonies

Materials and Chemicals Required

Soybean-casein digest agar, Distilled water, Autoclave, Disposable Petri plates, Colony counter.

Preparation of Medium

Prepare Soybean-casein digest agar and dispense in appropriate quantities. Sterilize, clean surface of working area with a suitable disinfectant. Mark clearly the petri plates identifying sample, sample unit, dilution and date of inoculation.

Preparation of Dilutions

Prepare a 1:10 dilution of the sample such as 1 g into 9 ml of the required diluents. Prepare succeeding decimal dilutions as required, using a separate sterile pipette for making each transfer. Shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

Plating

Agitate each dilution bottle to resuspend material that may have settled out during preparation. Moulds should be enumerated by a surface spread-plate technique rather than with pour plates, Pour 15 mL of tempered agar into each plate and allow it to solidify. Pour 1 ml of the diluted sample on top of the agar and spread evenly with the help of sterile L-rod. Plates should be poured and inoculated not more than 15 min after preparation of dilutions.

Incubation

Incubate plates undisturbed in an upright position at 22 to 25 °C for 3-5 days. Incubate plates in the dark. Normally, count colonies on plates after 5 days. Examine on the third day and if mould colonies are numerous, count them and then count again on the fifth day, if possible.

Counting Colonies

{Colony count (CFUs) on an agar plate} x {Total dilution of tube (used to make plate for colony count)}

Detection of Total Yeast Count

Materials and Chemicals Required

Sabouraud dextrose agar, Distilled water, Autoclave, Disposable Petri plates, Colony counter.

Preparation of Medium

Prepare Sabouraud dextrose agar and dispense in appropriate quantities. Sterilize Clean surface of working area with a suitable disinfectant. Mark clearly the petri plates identifying sample, sample unit, dilution and date of inoculation.

Preparation of Dilutions

Prepare a 1:10 dilution of the sample such as 1 g into 9 mL of the required diluents. Prepare succeeding decimal dilutions as required, using a separate sterile pipette for making each transfer. Shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

Plating

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Counting Colonies

{Colony count (CFUs) on an agar plate} x {Total dilution of tube (used to make plate for colony count)}

RESULTS AND DISCUSSION

Microorganisms live all around us, and they are capable of surviving in a wide range of environmental conditions. They are a necessary part of our world and perform a variety of useful functions that is fermentation, digestion, nutrition and recycling. As per the latest Food Safety and Standards Act, 2006, every food product has to be tested and approved before it is put up for sale in the market. Increases in food production and the ever present threat of food contamination from microbiological and chemical sources have led the food industry and regulators to pursue rapid, inexpensive methods of analysis to safeguard the health and safety of the consumer. It can also cause food borne illness (Aguilera and David, 1990).

Oriens® Wheatgrass is a wheatgrass powder encapsulated and used as a food supplement. During processing of Oriens Wheatgrass, initially the qualities of the received raw materials were analyzed. Then, the raw

material was sifted, blended, filled and packed. During the process of handling, it is very important to ensure the microbial safety of the product. The microbial growth of the Oriens® Wheatgrass was estimated by Indian Pharmacopoeia method.

Due to their natural origin, questions on microbiological quality arise more often for herbal products (ICH, 2003). For this reason, particularly in case of new applications, a detailed microbiological investigation program is often set up for herbal medicinal products. The microbial analysis of the Oriens® Wheatgrass was given in Table 1.

Table 1: Microbial Testing of Oriens® Wheatgrass

Oriens Wheatgrass – VNL18-021			
S. No.	**Parameters**	**Specification**	**Results**
1	Total Bacterial Count	10^5 CFU/g	<150 CFU/g
2	Total Yeast and mold	10^3 CFU/g	< 200 CFU/g
3	*Escherichia coli*	Absent	Absent
4	*Salmonella sp.*	Absent	Absent
5	*S. aureus*	Absent	Absent

In this study, five different species of microorganism which were total bacterial count, total yeast and mould count, *E. coli, Salmonella sp*. and *Staphylococcus aureus* were tested to identify the microbial growth.

The total bacterial count of Oriens® Wheatgrass was found to be <150 CFU/g which is under the limit. The total yeast and mold was <200 CFU/g, it is under the permissible limit. The pathogenic microorganism such as *Escherichia coli, Salmonella sp.* and *S. aureus* were found to be absent. This ensures that Oriens® Wheatgrass is safe for consumption and upon observation no microbial growth were observed. It determined that the product was developed under Good manufacturing practice.

CONCLUSION

Consumers can easily acquire pathogenic microorganisms by consuming contaminated products. The good handling must be carried out starting from raw materials to finished products. The results we found from our study were there was no abnormal growth of total bacterial count and yeast and mold count in Oriens® Wheatgrass. It was under the limits and specification. Moreover, there was an absent of pathogenic microorganism. The resultant finding indicated that the Oriens® Wheatgrass were processed under hygiene observance.

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