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Characterization of Microbes in Protein Supplements Available Commercially

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Authors' contributions

This work was carried out in collaboration among all authors. Author MA did all experimental works, managed the analyses of the study. Author MA managed the literature searches and the first draft of the manuscript. Authors AA and OAB designed the study, performed the statistical analysis, wrote the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Supplements have been used widely since several years and their production has greatly increased with many different types, e.g. (Vitamins, Collagen, Proteins. etc...), and for multiple uses. Supplements were made to complete the nutrients deficiency that people have, or to prevent the deficiency condition, and some are using it to boost their body with nutrients like the athletes. Protein is one of the most selling supplements in the market used as powder or drink, and also been added to many different foods and snacks to make it healthier. This paper involves a food safety and quality study to identify and characterize the microbial pathogens in protein powder supplements available commercially.

Keywords: Microbes; protein; supplements health.

1. INTRODUCTION

Protein powder is one of the most popular nutritional supplements that has being used by a variety of people nowadays, (Male, Female, athletic, and who had recent surgery also recommended for it) [1]. Protein supplement is an essential macronutrient helps building muscle,

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repair tissue, and make enzymes and hormones [2]. Using protein powder may also aid weight loss and help people tone their muscles [3,4,5]. There are many different types of protein powder, including animal-based: From animal sources e.g. (Milk, Egg, Beef etc.) and plant-based: From plant sources e.g. (Soy, Pea etc.). Improper handling and manufacturing of protein supplements brings or contaminate it. Such contaminated protein supplements are going to infect health human being and cause illness to the wrong term health defects. Here I have characterized and concluded how much and which microbes are contaminating the protein supplement by their Morphological and Biochemical using ISO/GSO standard methods. Plant proteins have more contamination than the animal-based protein supplements. *Baccilus cereus*, *E.coli* and Yeast and Mold were reported in plant-based protein supplements. Caution should be taken before choosing protein supplements. More study is needed to ascertain the reported result.

Use of protein supplements have been increased in current time as used by variety of persons [6]. Proteins are recommended as supplements as it help in building muscle, tissue repairing, in making of hormones and enzymes [7]. The protein supplements that we eat are free of microbes and contaminations, but due to improper handling and non-hygienic conditions these materials got infected which make it unhealthy [8]. Under the law, manufacturers of dietary supplements are responsible for making sure their products are safe before they go to market [9]. They are also responsible for determining that the claims on their labels are accurate and truthful [10,11]. Dietary supplement products are not reviewed by the government before they are marketed [12,13]. but FDA has the responsibility to act against any unsafe dietary supplement product that reaches the market. If FDA can prove that claims on marketed dietary supplement products are false and misleading, the agency may act also against products with such claims [14]. Due to this condition, we will make analysis by different methods for detection of specific pathogens that have high potential to be present in protein supplement, to make sure if it contains any of these pathogens.

2. MATERIALS AND METHODS

Samples: 10 different protein Samples were collected from the most famous and selling brands on the market, 7 types of microbe have been tested with each sample 5 times [15], ending with total of 350 samples tested.

Determination of microbes in protein: Petri plate method techniques, as described by FDA-BAM (Bacteriological analytical manual) was used to determine and analyze the microbes [16], and specific pathogenic bacteria which is have high potential to grow and present on the protein supplement as per the GSO standards [15], which include (Salmonella, Staphylococcus aureus, Total coliform, Escherichia coli, Bacillus cereus, Total bacterial count, Yeast & Mold).

Dehydrated media and supplements: Plate Count Agar (PCA), Potato Dextrose Agar (PDA), Baird Parker Agar (BPA), Violet Red Bile Agar (VRBA), Eosin Methylene Blue Agar (EMB), Bacillus Cereus Agar (BCA), Xylose Lysine Deoxycholate Agar (XLD), Tetrathionate Broth (TTB), Rappaport Vassiliadis Broth (RV), Xylose Lysine Tergitol 4 Agar (XLT4), Brilliant Green Sulfa Agar (BGS), Triple Sugar Iron Agar (TSI), Buffered Peptone Water (BPW), Egg Yolk Emulsion, Polymyxin B Sulphate, Egg Yolk Tellurite Emulsion, Iodine solution.

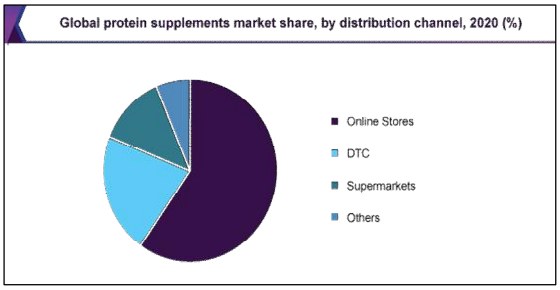


Fig. 1. The U.S. protein supplement market size by product

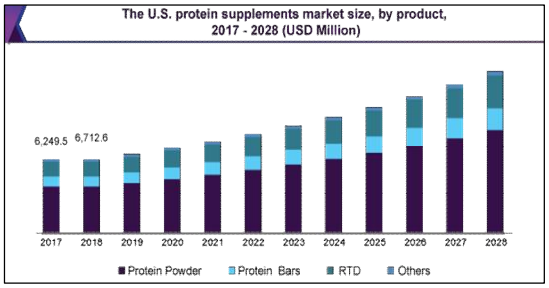
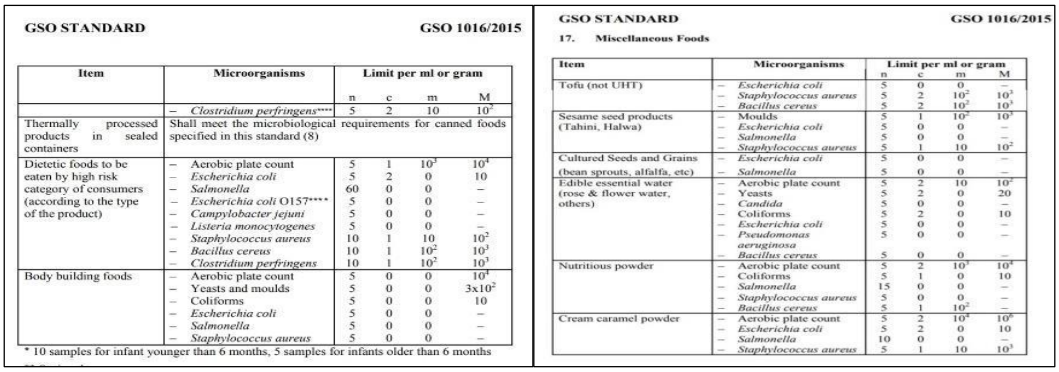


Fig. 2. Global protein supplements market share by distribution channel



GSO STANDARD GSO 1016/2015

Item	Microorganisms	Limit per ml or gram			
		n	c	m	M
	– *Clostridium perfringens*****	5	2	10	10^2
Thermally processed products in sealed containers	Shall meet the microbiological requirements for canned foods specified in this standard (8)				
Dietetic foods to be eaten by high risk category of consumers (according to the type of the product)	– Aerobic plate count	5	1	10^3	10^4
	– *Escherichia coli*	5	2	0	10
	– *Salmonella*	60	0	0	–
	– *Escherichia coli* O157****	5	0	0	–
	– *Campylobacter jejuni*	5	0	0	–
	– *Listeria monocytogenes*	5	0	0	–
	– *Staphylococcus aureus*	10	1	10	10^2
	– *Bacillus cereus*	10	1	10^2	10^3
	– *Clostridium perfringens*	10	1	10^2	10^3
Body building foods	– Aerobic plate count	5	0	0	10^4
	– Yeasts and moulds	5	0	0	$3x10^2$
	– Coliforms	5	0	0	10
	– *Escherichia coli*	5	0	0	–
	– *Salmonella*	5	0	0	–
	– *Staphylococcus aureus*	5	0	0	–

* 10 samples for infant younger than 6 months, 5 samples for infants older than 6 months

GSO STANDARD GSO 1016/2015

17. Miscellaneous Foods

Item	Microorganisms	Limit per ml or gram			
		n	c	m	M
Tofu (not UHT)	– *Escherichia coli*	5	0	0	–
	– *Staphylococcus aureus*	5	2	10^2	10^3
	– *Bacillus cereus*	5	2	10^2	10^3
Sesame seed products (Tahini, Halwa)	– Moulds	5	1	10^2	10^3
	– *Escherichia coli*	5	0	0	–
	– *Salmonella*	5	0	0	–
	– *Staphylococcus aureus*	5	1	10	10^2
Cultured Seeds and Grains	– *Escherichia coli*	5	0	0	–
(bean sprouts, alfalfa, etc)	– *Salmonella*	5	0	0	–
Edible essential water (rose & flower water, others)	– Aerobic plate count	5	2	10	10^2
	– Yeasts	5	2	0	20
	– *Candida*	5	0	0	–
	– Coliforms	5	2	0	10
	– *Escherichia coli*	5	0	0	–
	– *Pseudomonas aeruginosa*	5	0	0	–
	– *Bacillus cereus*	5	0	0	–
Nutritious powder	– Aerobic plate count	5	2	10^3	10^4
	– Coliforms	5	1	0	10
	– *Salmonella*	15	0	0	–
	– *Staphylococcus aureus*	5	0	0	–
	– *Bacillus cereus*	5	1	10^2	–
Cream caramel powder	– Aerobic plate count	5	2	10^4	10^6
	– *Escherichia coli*	5	2	0	10
	– *Salmonella*	10	0	0	–
	– *Staphylococcus aureus*	5	1	10	10^3

Fig. 3. GSO standard for microorganism's satisfactory limits

Samples were prepared using aseptic technique and diluted using a ratio of 1:10 dilution, then homogenized in the stomacher. 1 ml was transferred to petri plate then media was added [(pour method for TPC, Y.M, and Coliform), and (Spread method for Staph.a, and B.cereus)], Inoculation was in different methods (pour, spread, and streak) depend on the microbe been tested. Each microbe was tested x5, then Samples were kept till they dried/absorbed through agar. they were putted in the incubators at 35°C for 48h for bacteria, and 27°C for 120h for yeast and mold. Incubation time/temperature is depending on the media used and the microbe been tested [17,18,19,20,21,22].

Salmonella sample was prepared using (BPW) as pre-enrichment 25g/225ml and incubated at 35°C for 24h. sample was tested x5, then 0.1ml was transferred into 10ml RV broth test tubes and incubated at 42°C for 24h. After incubated was done, streaking technique was used to inoculate the sample into XLT and BGS agar plates, then incubated at 35°C for 24h [23].

After incubation time finished, samples were taken to determine the growth status and count it.

3. RESULTS

All protein samples showed satisfactory results in each test (Tables 1-6) which is within the count limit, except plant protein which showed an unsatisfactory results for (Yeast and Mold), and fairly satisfactory results for (*B.cereus*), and unsatisfactory results for (TPC) (Table 7).

All microbes showed satisfactory results with <1 count (Figs. 4-7), except *Bacillus cereus* in plant protein showed Fairly Satisfactory results with 50 CFU/gm (Fig. 8). Yeast and Mold in Plant protein was having unsatisfactory results with 300 CFU/mg (Fig. 9), TPC in plant protein was within limit but having a high count 1000 CFU/mg (Fig. 10).

Table 1. Results of concentrated protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number of samples tested
	Salmonella spp.	0	0		
	Staphylococcus aureus	0	0		
Concentrate protein powder	*Baccilus cereus*	0	0	35	35
	Coliform	0	0		
	E. coli	0	0		
	TPC (Total Plate Count)	0	0		
	Y and M (Yeast and Mold)	0	0		
Total		0	0	35	35

Table 2. Results of isolated protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number of samples tested
Isolated protein powder	*Salmonella spp.*	0	0	35	35
	Staphylococcus aureus	0	0		
	Baccilus cereus	0	0		
	Coliform	0	0		
	E. coli	0	0		
	TPC (Total Plate Count)	0	0		
	Y and M (Yeast and Mold)	0	0		
Total		0	0	35	35

Table 3. Results of hydrolyzed protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number of samples tested
Hydrolyzed protein powder	*Salmonella spp.*	0	0	35	35
	Staphylococcus aureus	0	0		
	Baccilus cereus	0	0		
	Coliform	0	0		
	E. coli	0	0		
	TPC (Total Plate Count)	0	0		
	Y and M (Yeast and Mold)	0	0		
Total		0	0	35	35

Table 4. Results of casein protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number Of samples tested
Casein protein powder	*Salmonella spp*.	0	0	35	35
	Staphylococcus aureus	0	0		
	B.cereus	0	0		
	Coliform	0	0		
	E. coli	0	0		
	TPC (Total Plate Count)	0	0		
	Y and M (Yeast and Mold)	0	0		
Total		0	0	35	35

Table 5. Results of complex protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number Of samples tested
	Salmonella spp.	0	0		
	Staphylococcus aureus	0	0		
Complex	*B.cereus*	0	0	35	35
protein	*Coliform*	0	0		
powder	*E. coli*	0	0		
	TPC (Total Plate Count)	0	0		
	Y and M (Yeast and Mold)	0	0		
Total		0	0	35	35

Table 6. Results of whey synt protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number Of samples tested
	Salmonella spp.	0	0		
	Staph.a	0	0		
	B.cereus	0	0		
Whey	Coliform	0	0	35	35
synt	E. coli	0	0		
protein	TPC	0	0		
powder	Y.M	0	0		
Total		0	0	35	35

Table 7. Results of mass gain tr protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number Of samples tested
	Salmonella spp.	0	0		
	Staphylococcus aureus	0	0		
|Mass gain	*B.cereus*	0	0	35	35
tr protein	*Coliform*	0	0		
powder	*E. coli*	0	0		
	TPC	0	0		
	Y.M	0	0		
Total		0	0	35	35

Fig. 4. *Salmonella* results on diagram. (All results were negative)

Table 8. Results of mass gain ser protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number Of samples tested
	Salmonella spp.	0	0		
	Staphylococcus aureus	0	0		
Mass gain				35	35
ser protein	*B.cereus*	0	0		
powder	*Coliform*	0	0		
	E. coli	0	0		
	TPC	0	0		
	Y.M	0	0		
Total		0	0	35	35

Table 9. Results of complex nit protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactory	Number Of samples tested
	Salmonella spp.	0	0		
	Staphylococcus aureus	0	0		
Complex				35	35
protein	*B.cereus*	0	0		
powder	*Coliform*	0	0		
	E. coli	0	0		
	TPC	0	0		
	Y & M	0	0		
Total		0	0	35	35

Table 10. Results of plant protein

Sample	Analysis	Unsatisfactory	Fairly satisfactory	Satisfactor y	Number of sample s tested
	Salmonella spp.	0	0		
	Staph.aureus	0	0		
	B.cereus	0	5		
Plant protein	*Coliform*	0	0	20	35
powder	*E. coli*	0	0		
	TPC	0	5		
	Y and M	5	0		
Total		5	10	20	35

Fig. 5. *Staphylococcus aureus* results on diagram. (All results were negative)

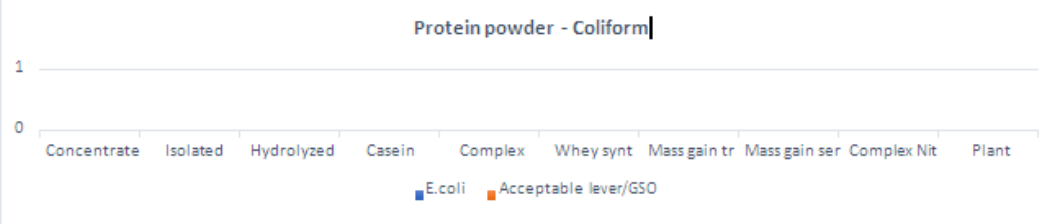


Fig. 6. Coliform results on diagram. (All results were negative)

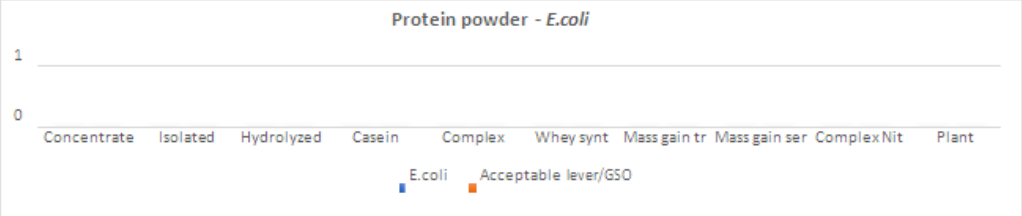


Fig. 7. *E. coli* results on diagram. (All results were negative)

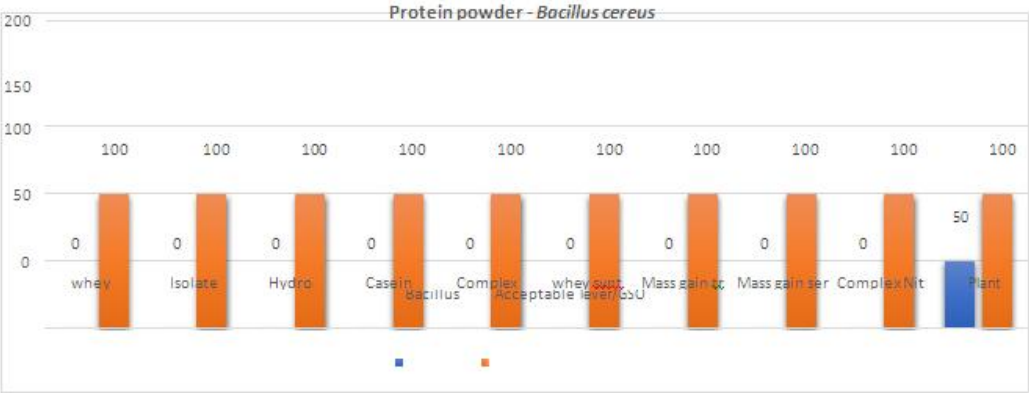


Fig. 8. *B.cereus* results on diagram. (Results were negative, except plant protein were little high positive counts but within the limit)

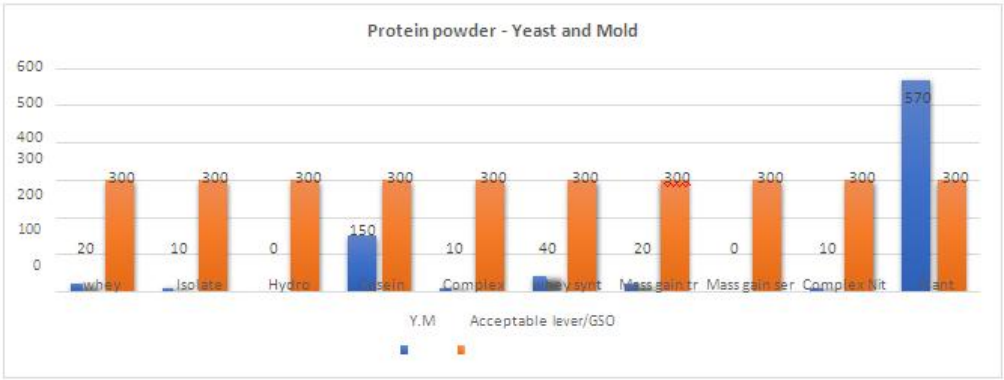


Fig. 9. Yeast and Mold results on diagram. (Counts were satisfactory in all proteins, except plant protein was unsatisfactory)

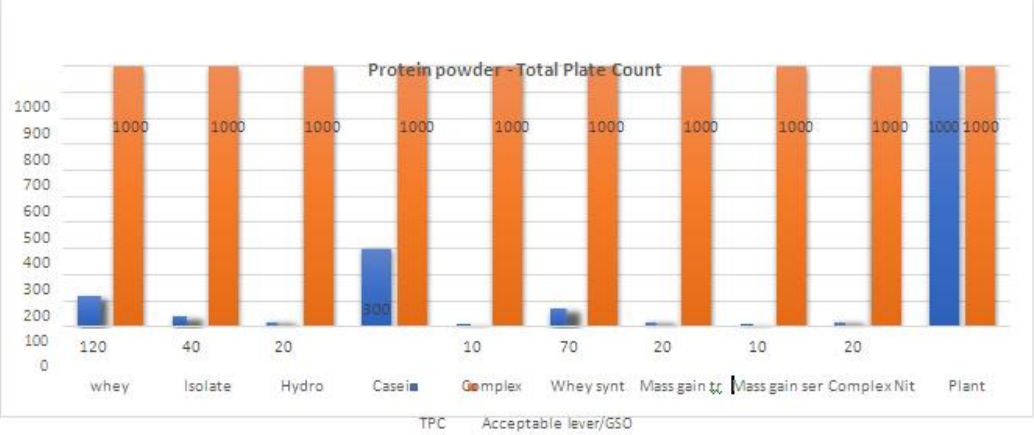


Fig. 10. TPC results on diagram. (Counts were satisfactory in all proteins, except plant protein was unsatisfactory)

4. DISCUSSION

In previous studies on different types of protein powder, plant-based protein and seed powder were having bacterial pathogens e.g. (B.cereus and C.perfringens) [23], and other studies showed that toxins and heavy metals were present in significant quantities in 53 leading brands, which considered as a food safety contamination [12,23,24,25]. Out of 350 samples from different proteins were tested in this study, 20 plant protein samples showed questionable results of (*B.cereus*), and having high count of (TPC) which means that there is some contamination appear and the results of (Y.M) were above the limit and showed an unsatisfactory results. Y.M will not just spoilage the product but also can spread toxins. Y.M produces spores that can survive in extreme conditions and it produce toxins, like mycotoxin which can be serious health threat and it's ranged from acute poisoning to long term such as cancer and immune deficiency. Manufacturers should be more aware for health safety of their products, especially that most of their product will be consumed as a milk shake, so no heat or treatment will be exposure to the product once it's produced and distributed to the market.

5. CONCLUSION

Protein is one of the most selling supplements in the market used as powder or drink, and also been added to many different foods and snacks to make it healthier. This paper involves a food safety and quality study to identify and characterize the microbial pathogens in protein powder supplements available commercially.

6. RECOMMENDATIONS

Most of the animal-based protein powder supplements was having satisfactory results which is mean that most of these common companies are following good manufacturer practices with a good food safety condition to produce supplement products in proper and hygienic way with a good Quality.

Plant proteins have less quality and food safety than the other proteins, maybe one of the reasons is the soil, which is having a lot of contamination factors, so it can contaminate the product or the environment of the processing and production area if it's handled with poor and improper hygienic way.

We need high inspection for the supplement manufacturers to monitor their products, to avoid any contamination or health risk cases.

More study needs to be done on plant-based protein and the other supplements with most of the leading brand in the market, and to test it for more different contamination test e.g. (chemical and allergy contamination) not just focusing only on microbial contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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