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Microbiological properties of nutribar incorporated with legumes

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Abstract
The goal of the current study was to developed nutribars that contained varied amounts of legumes. And to assess the Microbiological analysis of nutribar. According to the study, the average total plate count was highest in T0, T4 and T9 with value of 2.28×10^2 and lowest in T8 with value of 2.25×10^2. The average yeast and mould count was highest in T12 with value of 2.00×10^2 and lowest in T3 with value of 1.90×10^2. The average sacchrolytic, lipolytic and proteolytic bacteria count in all nutribar samples was NIL. The mean value of control (T0) is 2.28. The above table also showing that treatment combination (T0) & (T8) contains highest and lowest total plate count (10^2 cfu/ml) than the other treatments respectively. The mean value of control (T0) is 1.91. The treatment combination (T12) & (T0) contains highest and lowest total yeast and mould count (10^2 cfu/ml) than the other treatments respectively. Because of treatments and replication, the ANOVA table up top shows that the F. Cal. Value is greater than the F. Tab. value at a 5% level of significance on each d.f. Additionally, the aforementioned table shows a significant difference ($p \leq 0.05$) between various therapies.

Keywords: Antioxidant, chickpea, human health, legumes, nutribar, tulsi

1. Introduction
Nutri-Composite Bars are a good, wholesome snack. Sales of ready-to-eat foods that sate hunger and offer a balance of nutrients (protein, fat, minerals, vitamins, calories, and carbohydrates) are constantly increasing (King, 2006; Ryland *et al.*, 2011; Wyatt, 2011) [4, 7, 12]. Nutribar was initially marketed as an energy drink for athletes.

Nutri bars are a frequent meal containing a combination of ingredients that work well to enhance nutrition for all age groups, while they are typically recommended for consumption by women (who are pregnant, nursing, or trying to get pregnant). Along with its unique nutritional attributes, Nutri Bar also has certain medicinal properties including demulcent, carminative, laxative, lactogenic, and rubefacient.

The moong bean is a remarkable short-season, summer-growing legume that is widely grown throughout the tropics and subtropics (*Vigna radiata* L.). Some countries, notably Bangladesh, Pakistan, and India, frequently eat moong beans. It is a little, oblong bean with a lot of fibre. The green moong bean turns yellow after the husk is removed. It helps treat food poisoning caused by a number of things, such as mushrooms and herbal remedies. The moong bean helps people lose weight. There are good concentrations of manganese, potassium, magnesium, folate, copper, zinc, and vitamin B sources. The moong bean is a substantial food that is starchy, high in protein, and contains fibre. It lowers blood pressure and cholesterol levels. The moong bean combats a number of chronic illnesses, including diabetes, cancer, heart disease, and obesity. The following nutrients can be found in one cup of cooked moong beans (percentages based on the RDAs for a typical adult female): 321 micrograms of folate (100%), 97 milligrammes of magnesium (36%), 0.33 milligrammes of vitamin B1 thiamine (36%), and 0.6 milligrammes of manganese (33%). 212 calories, 14 grammes of protein, 15 grammes of fibre, 1 gramme of fat, and 4 grammes of sugar are all found in this dish. Along with 55 milligrammes of calcium (5%), 0.13 milligrammes of vitamin B6 (11%), and 0.8 milligrammes of pantothenic acid (vitamin B5), 7 milligrammes of zinc (24%) are also present (Singh *et al.*, 2020) [8].

The annual legume chickpea, commonly referred to as chick pea, is a member of the Fabaceae family and subfamily Faboideae. Among the many names for its several types are gramme, Bengal gramme, garbanzo, garbanzo bean, and Egyptian pea. With a high concentration of protein, dietary fibre, folate, and several dietary minerals including iron and phosphorus per 100 grammes (20% or more of the Daily Value), chickpeas are a nutrient-dense food (El-Adawy, 2002) [1].

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The low concentrations of magnesium, zinc, and vitamin B6 provide 10-16% of the DV. When compared to reference values established by the World Health Organization and the United Nations Food and Agriculture Organization, proteins in cooked and germinated chickpeas are high in essential amino acids such lysine, isoleucine, tryptophan, and total aromatic amino acids. Cooked chickpeas contain 164 calories per 100 grammes (690 kJ). Cooked chickpeas contain 60% water, 27% carbohydrates, 9% protein, and 3% fat (table). Linoleic acid makes up 43% of the total fat, and unsaturated fatty acids account for 5% of the lipid composition (Jukanti *et al.*, 2012) [3].

A native species of legume from East Asia, the soybean, also referred to as the soya bean, is widely farmed for its edible bean. Most beans have a protein content of 20 to 25%, whereas soybeans have a protein content of about 40%. Protein can be found in soybeans. Typically, soybeans have 18–20% oil. Carbohydrates make up about 30% of it. It offers a significant amount of dietary fibre and has been shown to reduce the incidence of colon cancer and other diseases (Mateos-Aparicio *et al.*, 2008) [6]. Jaggery, sometimes referred to as "Gur," is a natural, unadulterated, conventional, complete sugar made by concentrating sugarcane juice that has not been treated with any preservatives. Jaggery, one of the oldest sweeteners known to man, is a mainstay of the peasant diet in many countries (Mandal *et al.*, 2006) [5]. The hues of jaggery range from light brown to dark golden. Tulsi leaves include antioxidants such as Circilinol, Circimartin, Isothymusin, Apigenin, and Rosameric Acid that can scavenge free radicals (Tewari *et al.*, 2021) [9]. (Verma, 2016) [11]. People are becoming more health conscious today, and as a result, they prefer items with additional value because they have more nutritional content. Flavonoids and caffeic acid are among the antioxidants present in sprouted moong beans. It lowers levels of harmful LDL cholesterol, lowers heart disease, and is rich in potassium, magnesium, and fibre, which may lower blood pressure. The folate in moong beans helps to promote a healthy pregnancy and boosts a baby's metabolism and immunity.

The soybean is one of the many and inexpensive sources of protein. Animals and humans alike frequently consume soybeans in many different places of the world. Soybeans are a fantastic source of protein for people with diabetes because they contain no carbs. Soybean seeds contain 17% oil, 63% meal, and 50% protein. Chickpeas are rich in protein. It regulates weight, enhances digestion, and prevents chronic diseases including diabetes, cancer, and heart conditions. It also reduces blood sugar levels. Circilinol, Circimartin, Isothymusin, Apigenin, and Rosameric Acid are antioxidants found in Tulsi leaves. Nutrition bars, often known as nutribars, have various benefits. The oldest known medicinal plant in India is tulsi,

which has a wide range of positive effects on human health, including the prevention of viral fever and cough (Tewari *et al.*, 2020) [12]. They start out being quite useful and small enough to fit in a desk drawer, gym bag, handbag, backpack, or the glove box of a car. Second, most nutribars are heavily fortified with calcium, protein sources comparable to a small chicken breast, vitamins and minerals (just like a bowl of cereal), and fibre. They are undoubtedly a much better choice than a candy bar, box of cookies, or bag of chips from a vending machine for a quick, on-the-go lunch or snack. Jaggery aids in easing joint pain, blood purification, boosting immunity, preventing anaemia, managing blood pressure, and preventing constipation. Additionally, it functions effectively as a binding agent.

This present research work was carried out to prepare nutribar incorporated with different ratios of legumes. To evaluate the Microbiological analysis of nutribar incorporated with legumes.

2. Material and Methods
The experiment 'Microbiological properties of Nutribar incorporated with legumes' was carried out in research laboratory of Warner college of dairy technology from Sam Higginbottom University of Agriculture, Technology & Science, Prayagraj.

2.1 Selection of ingredients
Sprouted whole moong bean, roasted chick pea, sprouted soya bean, jaggery, ghee, tulsi leaves will be procured from the local market of Prayagraj.

2.2 Preparation of raw material
Selection of moong beans, soyabean, and, chick pea, clean the three legumes, then soaking process will be start moong beans for (8 hrs at room temperature),soyabean for (24 hrs at room temperature),chickpea for (8 hrs at room temperature) then drain the excess water of moong beans and soyabean, and surface drying for chick pea, then germinate the moong bean and soyabean for 24 hrs at room temperature, and for chick pea roasting process will be done at 250 degree C (1-2 Min).The whole moong bean, soya bean, chick pea will be roasted separately, Then the tulsi leaves will be dried in a drier for 4 hours and made into a powder.

The sprouted whole moong bean, soya bean, chick pea, tulsi leaves will be mixed properly in a bowl. Then ghee is added in a pan, add crushed jaggery into the pan. After melting the jaggery add all the ingredients which will be mixed in a bowl and roasted, mix it properly till the mixture become thick. After that on the plate apply the ghee and grease it and put the mixture on it and keep it for set in room temperature and cut into a desire piece with the help of moulder.
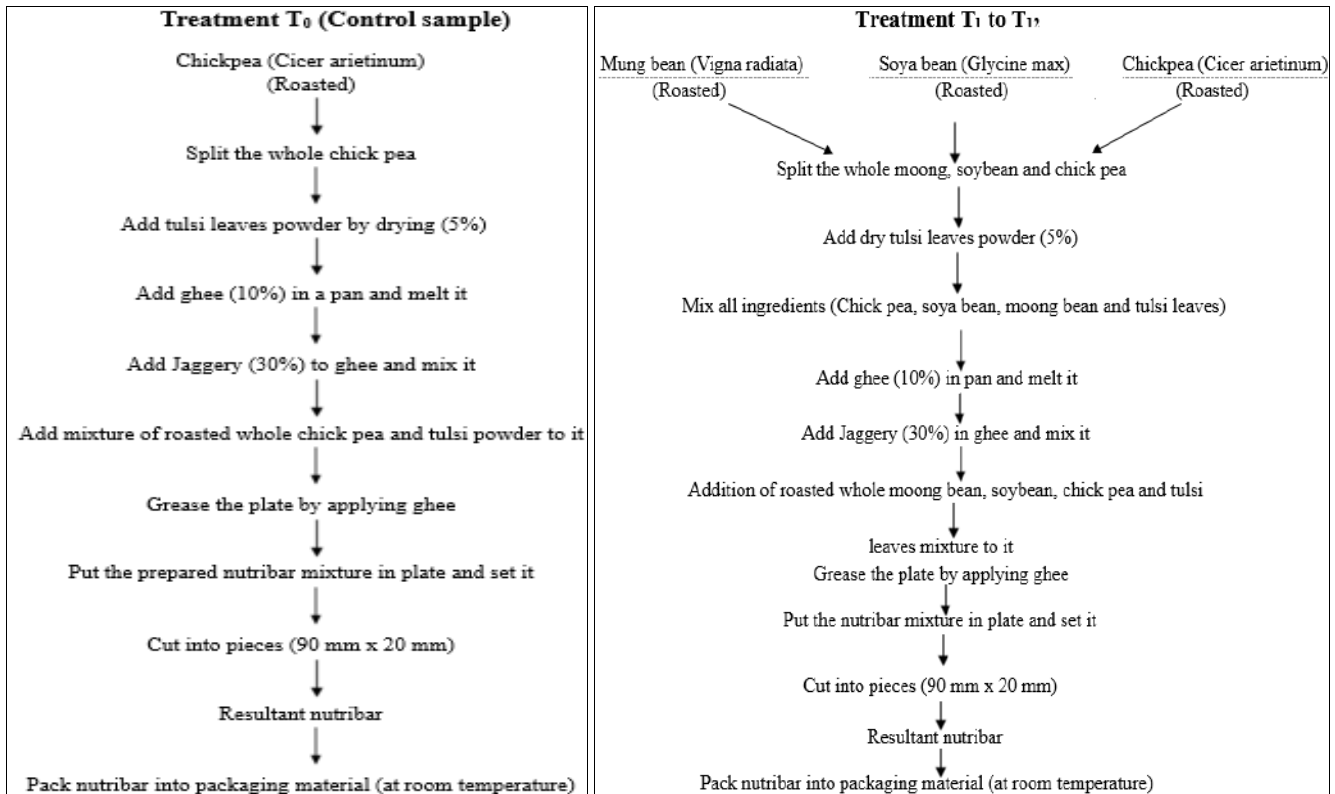
Plan of work

Treatment T_0 (Control sample)

Chickpea (Cicer arietinum) (Roasted)
↓
Split the whole chick pea
↓
Add tulsi leaves powder by drying (5%)
↓
Add ghee (10%) in a pan and melt it
↓
Add Jaggery (30%) to ghee and mix it
↓
Add mixture of roasted whole chick pea and tulsi powder to it
↓
Grease the plate by applying ghee
↓
Put the prepared nutribar mixture in plate and set it
↓
Cut into pieces (90 mm x 20 mm)
↓
Resultant nutribar
↓
Pack nutribar into packaging material (at room temperature)

Treatment T_1 to T_{12}

Mung bean (Vigna radiata) (Roasted)   Soya bean (Glycine max) (Roasted)   Chickpea (Cicer arietinum) (Roasted)
↓
Split the whole moong, soybean and chick pea
↓
Add dry tulsi leaves powder (5%)
↓
Mix all ingredients (Chick pea, soya bean, moong bean and tulsi leaves)
↓
Add ghee (10%) in pan and melt it
↓
Add Jaggery (30%) in ghee and mix it
↓
Addition of roasted whole moong bean, soybean, chick pea and tulsi leaves mixture to it
↓
Grease the plate by applying ghee
↓
Put the nutribar mixture in plate and set it
↓
Cut into pieces (90 mm x 20 mm)
↓
Resultant nutribar
↓
Pack nutribar into packaging material (at room temperature)

Table 1: Treatment Combination

Treatment	Composition of different Legumes (%)		
	Mung bean	Soyabean	Chickpea
T_1	85	10	5
T_2	80	15	5
T_3	75	20	5
T_4	70	25	5
T_5	80	10	10
T_6	75	15	10
T_7	70	20	10
T_8	65	25	10
T_9	75	10	15
T10	70	15	15
T11	65	20	15
T12	60	25	15

No. of treatments: 12+1
No. of replications: 5
No. of sample: 65

Sensory Evaluation
The nutribar was evaluated for sensory attributes by semi-trained panel by using 9- point Hedonic scale.

Statistical Analysis
The data obtained was statistically analysed by using factorial design and critical difference (C.D.) techniques (Imran and Coover, 1983) [2].

Microbial analysis of final prepared nutribar

Table 2: showing the mean of microbial analysis of final prepared nutribar

Treatment	Total plate count (10^2 cfu/mL)	Yeast and mould count (10^2 cfu/mL)	Sacchrolytic bacteria count (10^4 cfu/mL)	Lipolytic bacteria count (10^4 cfu/mL)	Proteolytic bacteria count (10^4 cfu/mL)
T_0	2.28	1.91	NIL	NIL	NIL
T_1	2.26	1.93	NIL	NIL	NIL
T_2	2.27	1.92	NIL	NIL	NIL
T_3	2.26	1.90	NIL	NIL	NIL
T_4	2.28	1.91	NIL	NIL	NIL
T_5	2.26	1.93	NIL	NIL	NIL
T_6	2.27	1.92	NIL	NIL	NIL
T_7	2.26	1.94	NIL	NIL	NIL
T_8	2.25	1.96	NIL	NIL	NIL
T_9	2.28	1.97	NIL	NIL	NIL
T_{10}	2.27	1.99	NIL	NIL	NIL
T_{11}	2.26	1.98	NIL	NIL	NIL
T_{12}	2.27	2.00	NIL	NIL	NIL

The average total plate count was highest in T0, T4 and T9 with value of 2.28 X 10^2 and lowest in T8 with value of 2.25 X 10^2. The average yeast and mould count was highest in T12 with value of 2.00 X 102 and lowest in T3 with value of 1.90 X 10^2. The average sacchrolytic, lipolytic and proteolytic bacteria count in all nutribar samples was NIL.

Table 3: Table showing total plate count (10^2 cfu/ml) of final prepared Nutribar

Treatments	R1	R2	R3	R4	R5	Mean
T_0	2.95	1.80	2.57	1.39	2.69	2.28
T_1	3.25	1.71	3.03	1.31	2.00	2.26
T_2	1.72	3.26	1.32	2.01	3.04	2.27
T_3	2.60	1.30	2.84	1.48	3.08	2.26
T_4	3.50	1.81	2.52	1.65	1.92	2.28
T_5	2.81	1.27	3.21	2.52	1.49	2.26
T_6	2.94	1.79	2.56	1.38	2.68	2.27
T_7	3.25	1.71	3.03	1.31	2.00	2.26
T_8	1.70	3.24	1.30	1.99	3.02	2.25
T_9	3.62	1.32	2.86	0.50	3.10	2.28
T_{10}	2.49	1.80	3.51	1.64	1.91	2.27
T_{11}	2.81	1.27	3.21	2.52	1.49	2.26
T_{12}	2.61	1.31	2.85	1.49	3.09	2.27
Mean	2.79	1.81	2.68	1.63	2.42	2.27
Minimum	1.70	1.27	1.30	0.50	1.49	2.25
Maximum	3.62	3.26	3.51	2.52	3.10	2.28
F- test				NS		
S. Ed. ($\pm$)				0.441		
C. D. (P = 0.05)				0.880		

The above table is showing that the mean value of control (T_0) is 2.28. The above table also showing that treatment combination (T_0) & (T_8) contains highest and lowest total plate count (10^2 cfu/ml) than the other treatments respectively.

Table 4: Table showing ANOVA for total plate count (10^2 cfu/ml) in final prepared Nutribar

ANOVA						
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result
Replication	4	13.9830	3.4957	7.181	2.57	S
Treatment	12	0.0054	0.0004	0.001	1.96	NS
Error	48	23.3678	0.4868	-	-	-
TOTAL	64	37.3562	-			-

The above ANOVA table is showing that the F. Cal. Value is higher than the F. Tab. value at 5% significant level on their respective d.f. due to replication. The above table also showing significant difference ($p<0.05$) between different treatments.
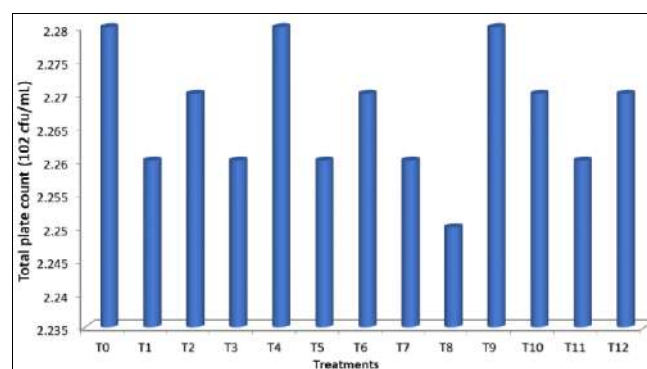

Fig 1: Graphical representation of total plate count (102 cfu/ml) of final prepared Nutribar

Table 5: Table showing total yeast and mould count (102 cfu/ml) of final prepared Nutribar

Treatments	R1	R2	R3	R4	R5	Mean
T_0	2.58	1.43	2.20	1.02	2.32	1.91
T_1	2.92	1.38	2.70	0.98	1.67	1.93
T_2	1.37	2.91	0.97	1.66	2.69	1.92
T_3	2.24	0.94	2.48	1.12	2.72	1.90
T_4	3.13	1.44	2.15	1.28	1.55	1.91
T_5	2.48	0.94	2.88	2.19	1.16	1.93
T_6	2.59	1.44	2.21	1.03	2.33	1.92
T_7	2.93	1.39	2.71	0.99	1.68	1.94
T_8	1.41	2.95	1.01	1.70	2.73	1.96
T_9	3.31	1.01	2.55	0.19	2.79	1.97
T_{10}	2.21	1.52	3.23	1.36	1.63	1.99
T_{11}	2.53	0.99	2.93	2.24	1.21	1.98
T_{12}	2.34	1.04	2.58	1.22	2.82	2.00
Mean	2.46	1.49	2.35	1.31	2.10	1.94
Minimum	1.37	0.94	0.97	0.19	1.16	1.90
Maximum	3.31	2.95	3.23	2.24	2.82	2.00
F- test				NS		
S. Ed. ($\pm$)				0.441		
C. D. (P = 0.05)				0.880		

The above table is showing that the mean value of control (T_0) is 1.91. The above table also showing that treatment combination (T_{12}) & (T_0) contains highest and lowest total yeast and mould count (102 cfu/ml) than the other treatments respectively.

Table 5: Table showing ANOVA for total yeast and mould count (102 cfu/ml) in final prepared Nutribar

ANOVA						
Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result
Replication	4	13.9830	3.4957	7.181	2.57	S
Treatment	12	0.0664	0.0055	0.011	1.96	NS
Error	48	23.3678	0.4868	-	-	-
Total	64	37.4172	-			-

The above ANOVA table is showing that the F. Cal. Value is higher than the F. Tab. value at 5% significant level on their respective d.f. due to replication. The above table also showing significant difference ($p<0.05$) between different treatments.
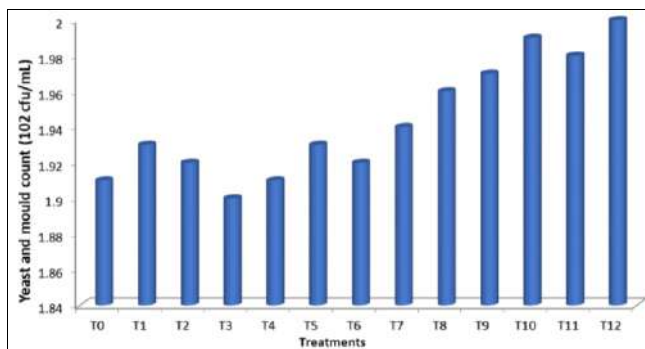

Fig 2: Graphical representation of total yeast and mould count (102 cfu/ml) of final prepared Nutribar

Conclusion

The average number of plates was 2.28×10^2 on average throughout T0, T4, and T9, and 2.25×10^2 on average across T8. T12 had the greatest average yeast and mould count (2.00×10^2) and T3 had the lowest average (1.90×10^2). All nutribar samples had a NIL average sacchrolytic, lipolytic, and proteolytic bacteria count. Control (T0) has a mean value of 2.28. The aforementioned table also demonstrates that treatment combinations (T0) and (T8) have the greatest and lowest total plate counts (10^2 cfu/ml), respectively, of all the treatments. Control (T0) has a mean value of 1.91. In comparison to the other treatments, the treatment combination (T12) & (T0) had the greatest and lowest total yeast and mould count (102 cfu/ml), respectively.

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