

Determining Vitamin B12 by Incorporating Chlorella Powder in Vegan Recipes

Research Article

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Abstract

Veganism is a diet that consists solely of plant-based foods and products; however, this diet lacks some nutrients, such as vitamin B12, because its sources are in animal-based foods, and because this diet, animal-based foods, and products have eliminated. People who follow this diet are tent to get vitamin B12 deficient. Chlorella is an alternative source of vitamin B12 for vegetarians and vegans. Therefore, this study aimed to determine vitamin b12 status by incorporating Chlorella powder into vegan recipes. Five vegan recipes were selected: Jeera Puri, Vegan Smoothie, Vegan Coconut Burfi, and Upma. In the statistical analysis, food products containing Chlorella were developed and evaluated. The sensory evaluation was carried out for jeera puri, vegan smoothie, Vegan coconut burfi and vegan gravy containing 1 g, 2g, 3g and 4g of chlorella powder. The premixes were stored for 3 months (premix) and 1 week (cooked), and it was observed that there was a significant difference within the groups (pO.05). The F-test revealed differences in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder at varying levels which are appearance- 0.025, taste- 0.016, texture- 0.179 and smell- 0.047 in the gravy premix. In addition, sample 2 of vegan gravy was less preferable, and sample 4 (3 g) was preferred by the panel members since as per taste was suitable for all. As a result, compared to all premixes, the Gravy premix was the best recipe as it stood out in all attributes such as appearance, texture, and smell in sensory evaluation.

Keywords: Vegetarianism; Veganism; Vegan recipes; European population; health impact; vitamin b12; vitamin b12 deficiency; alternate source; chlorella powder

Introduction

Vegetarianism and its different forms have become popular, including Lacto-vegetarianism, ovo- vegetarianism, lacto-ovo-vegetarianism, pescatarians and vegan [1].

Vegan diet is the strictest form of vegetarianism which forbids all animal origin, including dairy, eggs, meat, and poultry² and is replaced by plant-based foods. It is often motive by ethical, religious principles, environmental and health reasons³ but vegan diets tend to be lower in certain nutrients such as long-chain n-3 and vitamin B- 12 vitamin D, calcium, zinc, and vitamin B- 12 while being advanced in salutary fiber, magnesium, folic acid, vitamins C and E, iron, and phytochemicals [2,4].

Vitamin B12 (cobalamin) is a water-soluble vitamin which plays a crucial role in the nervous system, DNA, fatty acids, and myelin

production. Vitamin B12 functions as an enzyme cofactor. It is mostly found in flesh (especially beef, pork, and organ flesh (e.g., liver), flesh, milk and milk products [5]. Vitamin B12 deficiency is largely due to limited intake of animal origin foods or the malabsorption of vitamin. Vegans can meet their vitamin B12 demands from fortified foods or supplements. The most common form of vitamin B12 in salutary supplements is cyanocobalamin [6]. The most chemically stable and unnatural form of cobalamin is cyanocobalamin [6,7]. A supplement can be defined as any vitamin, mineral, chemical or botanical or herbal product that is added to the diet to prevent deficiencies but many scientists and health professionals agree that supplements can be taken under certain conditions and moral health but should not be replace complete and balanced foods that are necessary for a healthy diet [6]. Soy products such as miso, tempeh, shiitake (dry mushrooms), chlorella, spirulina, and nori (algae) are the vitamin b12 vegetarian sources⁸. Vitamin B12 deficiency is generally seen only

among vegetarians or vegans because vitamin B12 is present only in animal origin foods, but *C. vulgaris* could be an alternate source for the vitamin B12 status of vegan [3].

Chlorella is a green unicellular alga found in freshwater [9]. Dr. Martinus Willem Beijerinck discovered and described *Chlorella vulgaris* in 1890, a notorious microbiologist and botanist, it contains multitudinous nutrients and vitamins, including D and B12, that are absent in factory-deduced food sources [10]. Examinations of the vitamin B12 value of Chlorella in mortal health began in the early 1950s; Chlorella was first produced and consumed in Asia, substantially in Japan, and also commercially produced and distributed worldwide as a supplement [10].

Veganism is a particular diet that includes only plant-based foods and products, however, this diet lacks some nutrients such as vitamin B12 as its sources are in animal-based foods, and this diet, animal-based food, and products have eliminated this reason people who opt for this kind of diet are deficient of vitamin B12. *Chlorella vulgaris* will be an alternative source for improving the vitamin B12 status of vegetarians or vegans so the aim of this study is to determine the vitamin B12 status in chlorella and incorporate chlorella powder in vegan recipes.

Materials & Methods

Materials

The chlorella powder was purchased from Tonga herbs, Bhubaneswar, Odisha. All the raw ingredients for Vegan Jeera Puri, Vegan Smoothie, Vegan Coconut Burfi, Vegan Upma and Vegan Gravy premixes were purchased from Maheshwari store, Surat.

Method

This study is divided into three phases:

Phase 1: Preparation of 5 recipes

A) Outsourcing of chlorella powder:

The chlorella powder was purchased from Tonga herbs, Bhubaneswar, Odisha.

B) Preparation and standardization of vegan recipes with the incorporation of chlorella powder:

Standardization is a process developed to ensure the quality and quantity of food products and it is process of developing and implementing proper technical standards. Five vegan recipes were selected which were Vegan Jeera Puri, Vegan Smoothie, Vegan Coconut Burfi, Vegan Upma and Vegan Gravy and premixes were made out of them.

Phase 2: Sensory Evaluation

a) Selection of sensory panel members for organoleptic evaluation

30 panel members from different department were screened using threshold test in nutrition lab at Parul University. 9 semi trained panelist were selected.

Sensory evaluation was carried out for jeera puri, vegan smoothie,

vegan coconut burfi, and vegan gravy and vegan upma containing 0 gram, 1 gram, 2 grams, 3 grams and 4 grams of Chlorella powder.

b) Threshold test

Threshold is specific level below which a compound is not detectable and above which it can be detected by person with average sensory acuity. The purpose of the threshold test is to check the sensory ability of panel members. Each respective panel members was given 4 sets of samples containing 6 samples each with concentration 0% or pure water, 2%, 5%, 10%. Set 1 was salt water; set 2 was sugar water, 3 set as citric acid. The panel members were required to identify the solution and rate the sample in increasing intensity order.

Tools for organoleptic evaluation

Tools selected for this test for evaluation were Hedonic rating scale and different test.

c) Hedonic rating scale

Hedonic rating scale was used for visual and organoleptic evaluation. Panelist were asked to score each sample from 0 to 10 where 0 being the lowest and 10 being the highest on the basis of different attributes like

- Color
- Appearance
- Texture
- Taste

This test was conducted for assessment of all the attributes of all five food samples.

d) Statistical analysis:

The data was entered into Microsoft excel spreadsheet, verified and subjected to statistical analysis. Appropriate segregation of the data was done and the following calculations were performed.

- Mean and standard deviation
 - Percentages for hedonic scale
 - F' test were used to find out statistical significance between or within groups. (S1, S2, S3, S4, S5).
- ##### e) Nutrient Estimation:
- One of the best rated premixed was tested for the nutrient estimation from Gujarat Laboratory located at F-16,17, Madhavpura Market, Near Police Commissioner Office, Shahibaug, Ahmedabad – 380004.
 - Gravy was selected out of 5 recipes.
 - Total Fat, Carbohydrate and Protein with total energy
 - Micronutrient: sodium, dietary fibers, total sugars, Vitamin D, potassium, calcium, Iron and vitamin B12.

Phase 3: Self-life study:

To determined self- life study of Chlorella Powder incorporated vegan recipes.

- Selected top 5 panel members for shelf-life study
- Recipes were made with freshly made premixes
- The food products were incorporated with 0gm, 1gm, 2gms, 3gms and 4gms of chlorella powder.
- Sensory Evaluation was done

Results

In product development research, the main emphasis was on the development of optimal products concerning their sensory as well as specialized functional attributes. Most food products consist of numerous ingredients and their manufacture typically involves several different processing steps such as cooking, heating, baking, etc. every ingredient was important for preservation, cost and nutrient value. Therefore, the result of the study carried out under the present investigation with the interpretation findings. Five food products namely Vegan Upma, vegan Jeera Puri, Vegan Smoothie, Vegan coconut burfi and Vegan Gravy with Chlorella Powder were developed and evaluated. Sensory evaluation of five chlorella powder-incorporated food products was carried out by using a hedonic scale. Samples were developed for each food product and these samples were named S1, S2, S3, S4 and S5. S1 was standardized one and S2, S3, S4, S5 were the variation containing 1gm 2gms, 3gms and 4gms respectively. 9 trained panel members were selected and asked to score the food products based on various attributes on a hedonic scale.

As shown in the table 1 F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.099, taste-0.03, texture-0.100 and smell-0.005).

According to different tests, as shown in figure 1(a), Coconut Burfi, in the criteria of the appearance, sample1 (blank) showed similar acceptance with the sample 2 (1g), maximum acceptance at sample 5 (4g)

As shown in figure 1(b), Coconut Burfi, in the criteria of the taste, sample1 (blank) showed similar acceptance with the sample 2 (1g), and maximum acceptance at sample 5 (4g).

As shown in figure 1(c), Coconut Burfi, in the criteria of the texture, sample 4 (3g) showed similar acceptance with the sample 5 (4g), and maximum acceptance at sample 5 (4g) and minimum acceptance at sample 2(1g).

As shown in figure 1(d), Coconut Burfi, in the criteria of the smell showed sample1 (blank) has minimum acceptance at sample 2(1g), and maximum acceptance at sample 5 (4g).

As shown in the table 2 F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance- 0.095, taste-0.215, texture-0.774 and smell-0.109).

Table 1: Composite rating score for coconut burfi (Mean \pm Std. Deviation).

Attributes	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	F value
Appearance	2.00 \pm 1.225	2.00 \pm 1.225	2.56 \pm 0.527	2.89 \pm 0.782	3.11 \pm 1.269	2.101
Taste	1.56 \pm 0.726	1.56 \pm 0.726	2.00 \pm 0.500	2.56 \pm 0.882	2.78 \pm 0.972	4.716
Texture	2.11 \pm .782	2.22 \pm 1.093	2.67 \pm .866	3.00 \pm .707	3.00 \pm .866	2.088
Smell	1.67 \pm 0.707	2.00 \pm 0.866	2.22 \pm 0.667	2.67 \pm 0.866	3.11 \pm 0.928	4.370

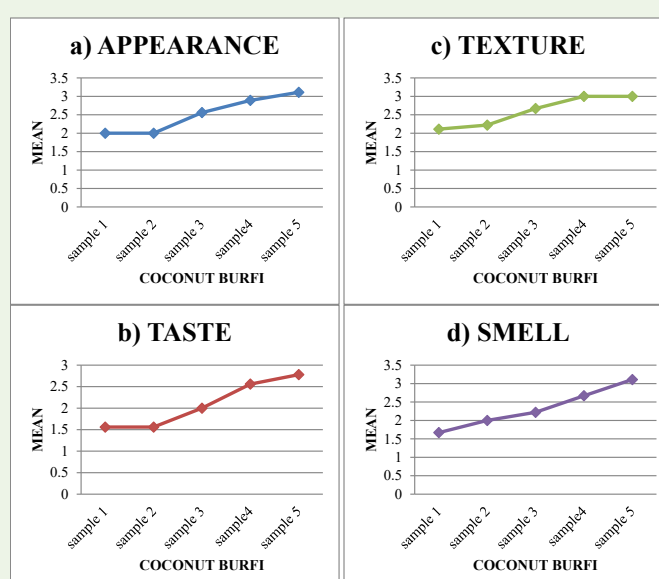
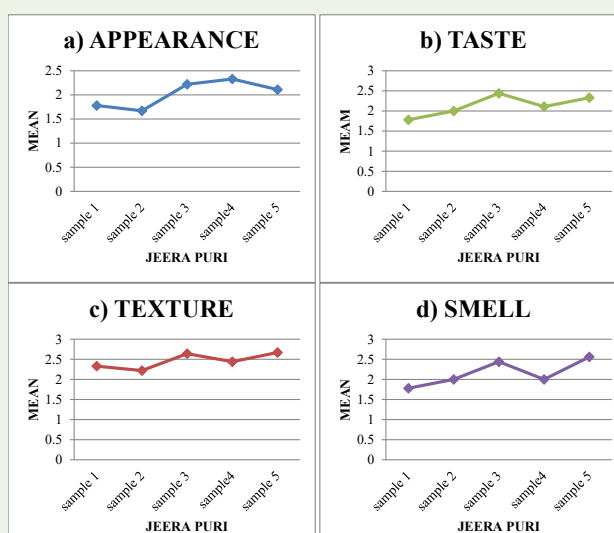


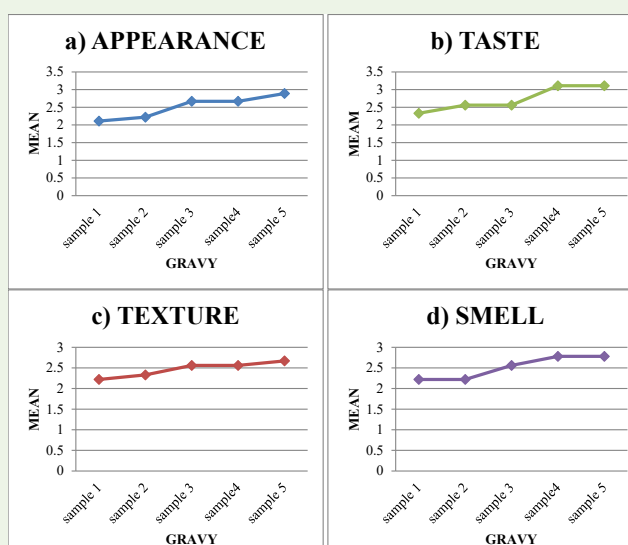
Figure 1: (a-d) scores for organoleptic attributes of coconut burfi with varying levels of incorporating chlorella powder sample 1, 2, 3, 4, 5 respectively.

Table 2: Composite rating score for Jeera Puri (Mean \pm Std. Deviation).

Attributes	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	F value
Appearance	1.78 \pm 0.441	1.78 \pm 0.441	2.22 \pm 0.441	2.33 \pm 0.500	2.11 \pm 0.782	2.127
Taste	1.78 \pm 0.667	2.00 \pm 0.707	2.44 \pm 0.527	2.11 \pm 0.601	2.33 \pm 0.707	1.520
Texture	2.33 \pm 0.866	2.22 \pm 0.833	2.67 \pm 0.866	2.44 \pm 0.726	2.67 \pm 1.118	0.448
Smell	1.78 \pm 0.667	2.00 \pm 0.707	2.22 \pm 0.441	2.00 \pm 0.500	2.56 \pm 0.726	2.029

**Figure 2:** (a-d) scores for organoleptic attributes of Jeera Puri with varying levels of incorporating chlorella powder sample 1, 2, 3, 4, 5 respectively.**Table 3:** Composite rating score for Gravy (Mean \pm Std. Deviation).

Attributes	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	F value
Appearance	1.44 \pm 0.527	2.00 \pm 0.866	2.22 \pm 0.972	2.78 \pm 0.833	2.33 \pm 0.866	3.138
Taste	1.67 \pm 0.707	1.89 \pm 1.054	2.22 \pm 0.972	2.56 \pm 0.882	2.89 \pm 1.054	2.462
Texture	1.78 \pm 0.833	1.89 \pm 0.928	1.89 \pm 1.167	2.56 \pm 1.236	2.78 \pm 1.093	1.655
Smell	1.78 \pm 0.833	2.00 \pm 1.118	1.89 \pm 0.928	2.78 \pm 1.202	2.89 \pm 1.054	2.311

**Figure 3:** (a-d) scores for organoleptic attributes of Gravy with varying levels of incorporating chlorella powder sample 1, 2, 3, 4, 5 respectively.

According to different tests, as shown in figure 2(a), Jeera Puri, in the criteria of the appearance showed minimum acceptance with the sample 2 (1g), maximum acceptance at sample 4 (3g).

As shown in figure 2 (b), Jeera Puri, in the criteria of the taste, showed minimum acceptance at sample 2 (1g), and maximum acceptance at sample 3 (2g).

As shown in figure 2(c), Jeera Puri, in the criteria of the texture, showed minimum acceptance at sample 2 (1g), and maximum acceptance at sample 3 (2g).

As shown in figure 2 (c), Jeera Puri, in the criteria of the smell, sample 2 (1g) showed similar acceptance with the sample 4(3g), and maximum acceptance at sample 5 (4g) and sample 3 (2g) and minimum acceptance at sample 2(1g) and sample 4 (3g).

As shown in the table 3 F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.025, taste-0.016, texture-0.179 and smell-0.047).

According to different tests, as shown in figure 3(a), Gravy, in the criteria of the appearance showed minimum acceptance with the sample 2 (1g), maximum acceptance at sample 3 (2g).

As shown in figure 3 (b), Gravy, in the criteria of the taste, showed minimum acceptance at sample 2 (1g) and sample 3(2g), and maximum acceptance at sample 4(3g) and sample 5(4g).

As shown in figure 3(c), Gravy, in the criteria of the texture, showed minimum acceptance at sample 2 (1g), and maximum acceptance at sample 3 (2g) and sample 4 (3g).

As shown in figure 3 (c), Gravy, in the criteria of the smell, showed maximum acceptance at sample 3 (2g) and minimum acceptance at sample 2(1g).

As shown in the table 4 F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.049, taste-0.007, texture-0.168 and smell-0.424).

According to different tests, as shown in figure 4(a), Upma, in the criteria of the appearance showed minimum acceptance with the sample 2 (1g), maximum acceptance at sample 3 (2g).

As shown in figure 4 (b), upma, in the criteria of the taste, showed minimum acceptance at sample 2 (1g) and sample 3(2g), and maximum acceptance at sample 4 (3g) and sample 5 (4g).

As shown in figure 4(c), Upma, in the criteria of the texture, showed minimum acceptance at sample 2 (1g), and maximum acceptance at sample 3 (2g) and sample 4 (3g).

As shown in figure 4 (c), Upma, in the criteria of the smell, sample 4 (3g) showed similar acceptance with the sample 5(4g), and maximum acceptance at sample 3 (2g) and minimum acceptance at sample 2(1g).

Table 4: Composite rating score for Upma (Mean \pm Std. Deviation).

Attributes	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	F value
Appearance	2.56 \pm 0.527	1.78 \pm 0.667	3.00 \pm 1.118	2.78 \pm 1.302	3.00 \pm 0.866	2.616
Taste	3.11 \pm 0.782	2.11 \pm 0.601	3.22 \pm 0.972	3.11 \pm 1.054	3.67 \pm 0.707	4.142
Texture	2.56 \pm 1.014	2.22 \pm 0.667	3.22 \pm 1.302	3.33 \pm 1.414	3.11 \pm 0.928	1.705
Smell	2.89 \pm 0.782	2.33 \pm 0.500	3.11 \pm 1.364	3.11 \pm 1.453	3.11 \pm 1.453	0.990

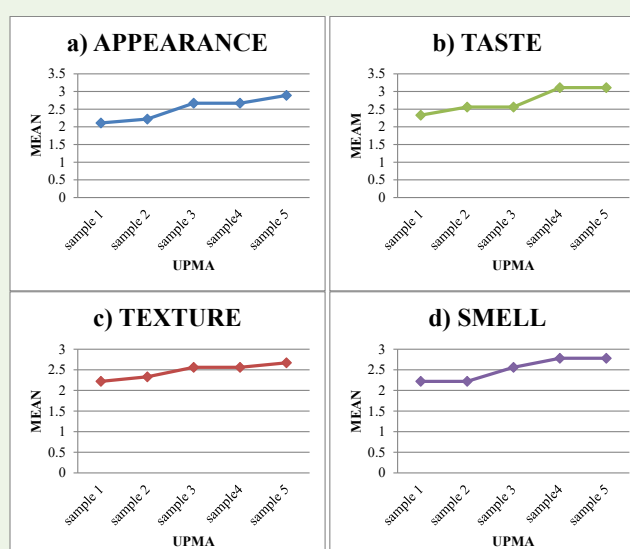


Figure 4: (a-d) scores for organoleptic attributes of Upma with varying levels of incorporating chlorella powder sample 1, 2, 3, 4, 5 respectively.

As shown in the table 5 F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.395, taste-0.245, texture-0.866 and smell-0.524).

According to different tests, as shown in figure 2(a), smoothie, in the criteria of the appearance showed minimum acceptance with the sample 2 (1g), maximum acceptance at sample 5 (4g) and similar acceptance between sample 3(2g) and sample 4(3g).

As shown in figure 2 (b), smoothie, in the criteria of the taste, showed minimum acceptance at sample 2 (1g) and sample 3(2g), and maximum acceptance at sample 4 (3g) and sample 5 (4g).

As shown in figure 2(c), smoothie, in the criteria of the texture, showed similar acceptance between the sample 3 (2g) and sample 4 (3g), and maximum acceptance at sample 5 (4g) and minimum acceptance at sample 2 (1g).

As shown in figure 2 (c), smoothie, in the criteria of the smell showed similar acceptance between the sample 4(3g) and sample 5 (4g), and maximum acceptance at sample 4 (3g) and sample 5 (4g) and minimum acceptance at sample 2(1g).

Phase 2: Nutrient analysis

Nutritional analysis selected the best recipe out of five coconut burfi, jeera puri, upma, smoothie and gravy.

The highest score of the incorporated chlorella powder vegan gravy was selected in the best recipe, with 2g chlorella powder vegan gravy with the highest score.

The test result selected samples are 200g of standard vegan gravy and 200g incorporated 2g chlorella powder.

Discussion

In this study different level of chlorella powder were added in the vegan recipes namely coconut burfi, gravy, jeera puri, upma and smoothie. Gravy was popular, however. They are associated with low vitamin b12 as they are vegan products which are lack in vitamin b12; As a result, an effort was made to make vitamin b12 rich foods by incorporating chlorella powder as vitamin b12 source at various levels, namely 1g, 2g, 3g and 4g. In present study gravy premix is made from all dried ingredients and added with chlorella powder will improved the vitamin b12 content in gravy. Sensory evaluation shows that sample 3 i.e. 2g of chlorella powder incorporated scored highest. Also has better score for taste compared to other attributes, that's why it was further send for nutrient analysis. According to the analysis, the content of vitamin b12 is sufficient.

All the vegan premixes was then packaged in zip lock pouches and kept at room temperature. It's cooking and sensory properties were tested at 90 days intervals. The premixes can be stored for 3 months (premix) and for 1 week (cooked) and observed that there is a significant difference within the groups which is ($p < 0.05$) and according to sensory evaluation result, the sample 3(2g) was more preferable and sample 5(4g) was less preferable by the panel members as the taste was suitable for all, the F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.025, taste-0.016, texture-0.179 and smell-0.047) in the gravy premix.

Furthermore, Coconut burfi was demonstrated that the sample 5(4g) was less preferable and sample 2 (1g) was more preferable by the panel members as the appearance was suitable for all, the F-test revealed significant difference in the organoleptic score in terms of

Table 5: Composite rating score for Smoothie (Mean \pm Std. Deviation).

Attributes	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	F value
Appearance	2.11 \pm 0.782	2.22 \pm 0.833	2.67 \pm 1.118	2.67 \pm 1.000	2.89 \pm 1.054	1.048
Taste	2.33 \pm 1.000	2.56 \pm 1.014	2.56 \pm 0.882	3.11 \pm 0.601	3.11 \pm 0.928	1.421
Texture	2.22 \pm 0.972	2.33 \pm 1.000	2.56 \pm 0.882	2.56 \pm 1.014	2.67 \pm 1.000	0.316
Smell	2.22 \pm 0.833	2.22 \pm 0.833	2.56 \pm 1.014	2.78 \pm 0.972	2.78 \pm 0.972	0.813

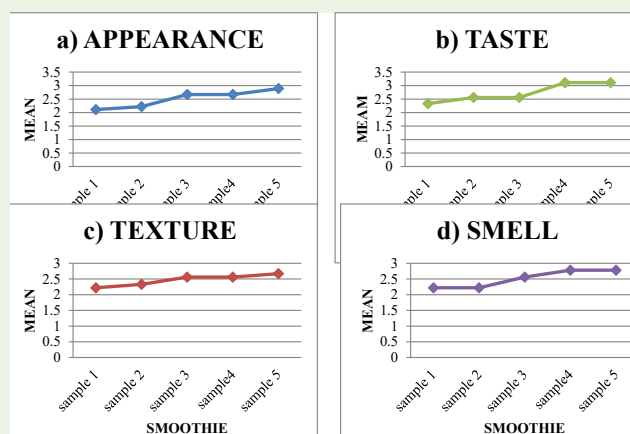


Figure 5: (a-d) scores for organoleptic attributes of smoothie with varying levels of incorporating chlorella powder sample 1, 2, 3, 4, 5 respectively.

Table 6: Nutrient profile Test reports.

Sr. No	Quality Characteristics	Test Results	Method
1	Energy kcal/100gm	414.60 kcal	GL/SOP/C-195
2	Total Fat gm /100gm	12.80g	GL/SOP/C-196
3	Trans Fat gm/100g	B.L.Q.(Q.L.=0.1)	FSSAI Manual of oils and fats method no.20.019
4	Saturated Fat gm/100g	2.25g	FSSAI Manual of oils and fats method no.20.019
5	Cholesterol gm/100g	B.L.Q.(Q.L.= 5.0)	AOAC 994.10
6	Carbohydrate gm/100g	63.51g	GL/SOP/C-195
7	Dietary fibers gm/100g	7.45g	GL/SOP/C
8	Protein gms/100gm	11.34g	GL/SOP/C-199
9	Sodium mg/100g	820.10mg	GL/SOP/I-055
10	Sugar and Total sugars g/100g	4.23g and 4.70g	GL/SOP/C-252
11	Vitamin D mcg/100g	B.L.Q.(Q.L.= 0.2)	GL/SOP/I-078
12	Calcium mg/100g	120.10mg	GL/SOP/I-055
13	Vitamin B12 mcg/100g	0.12 mcg	GL/SOP/I-078
14	Potassium mg/ 100g	390.12mg	GL/SOP/C-213
15	Iron mg/100g	4.60g	GL/SOP/I-055

appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance- 0.095, taste-0.215, texture-0.774 and smell-0.109).

Furthermore, observed that the sample 2 (1g) of Jeera Puri was less preferable and sample 3 (2g) was more preferable by the panel members as per the taste was suitable for all, the F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.095, taste-0.215, texture-0.774 and smell-0.109).

Upma, the sample 2 (1g) was less preferable and sample 4 (3g) was more preferable by the panel members as per the texture was suitable for all, the F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.049, taste-0.007, texture-0.168 and smell-0.424).

In addition to this, the sample 2 (1g) of smoothie was less preferable and sample 4 (3g) was more preferable by the panel members as per the appearance was suitable for all, the F-test revealed significant difference in the organoleptic score in terms of appearance, taste, texture and smell at all the levels of incorporation of chlorella powder (1g, 2g, 3g and 4g) at varying levels which is appearance-0.395, taste-0.245, texture-0.866 and smell-0.524).

Conclusion

Veganism is a diet that mainly consists of plant-based foods and products; however, this diet lacks some nutrients, such as vitamin B12, because its sources are in animal-based foods, and since this diet, animal-based foods, and products have eliminated this source

of vitamin B12, people who follow this diet are vitamin B12 deficient. So, chlorella powder as alternate source was incorporated in five vegan recipes including coconut burfi, smoothie, jeera puri, upma and gravy. Overall, compare to all premixes the gravy premix was the best recipe as it was more preferable by all the panel members because the gravy stands out in all the attributes such as appearance, taste, texture and smell in sensory evaluation.

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