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Effect of *Saccharomyces cerevisiae, Spirulina* and preservative supplementation to sweet bread quality in bakery

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Abstract
On the purpose of enhancing bread quality in bakery, we survey the effect of *Spirulina* supplementation to bread making. Our results show that bread supplemented this algae much more balance nutrient than control one. This supplementation also influences to bread volume and acidity. However, color in supplemented sample is inferior to control although it has super flavour. Our conclusion chooses sample M13 because of its high nutrient and sensory characteristics.

Keywords: Spirulina, supplementation, nutrient, sensory, bread

1. Introduction
Bread, the most common form of cereal intake in many countries, including Vietnam has been designated the staff of life, and rightly so, since it contains more nutrients per weight than meat, milk, potatoes, fruits, and vegetables. Bread may be made from various cereals, grains, and legumes.
Spirulina is common name of microalgae belonging to two genera *Spirulina* and *Arthrospira*. *Arthrospira* is a photosynthetic, filamentous, spiral-shaped, multicellular and blue- green micro alga. Cell division occurs by binary fission. As it contains chlorophyll a, like higher plants, botanists classify it as a micro alga belonging to Cyanophyceae class; but according to bacteriologists it is a bacterium due to its prokaryotic structure. Its chemical composition includes proteins (55%-70%), carbohydrates (15%-25%), essential fatty acids (18%) vitamins, minerals and pigments like carotenes, chlorophyll a and phycocyanin. Pigments are used in food and cosmetic industries. *Spirulina* is considered as an excellent food, lacking toxicity and have anticancer, antiviral, immunological properties and it also acts as a potent antioxidant. There has been a significant change in *Spirulina* functions under stress conditions (Shabana Kouser Ali and Arabi Mohammed Saleh, 2012)
Spirulina, now named *Arthrospira,* is a microscopic and filamentous cyanobacterium (blue-green alga) that has a long history of use as food. Early interest in *Spirulina* focused mainly on its rich content of protein, vitamins, essential amino acids, minerals, and essential fatty acids. *Spirulina* is 60-70% protein by weight and contains a rich source of vitamins, especially vitamin B12 and provitamin A (β-carotene), and minerals, especially iron. One of the few sources of dietary γ-linolenic acid (GLA), it also contains a host of other phytochemicals those have potential health benefits (Amha Belay, 2002). Furthermore this sea source has essential fatty acids such as Omega 6 and Omega 9. Presence of these compounds in *Spirulina* algae is its unique characteristic comparing to other supplements. Furthermore, this algae has various therapeutic benefits such as amplifying an immunity system, excretion of heavy metals, aiding the food digestion, ulcer repair, improving vision (Masoud Baghestani *et al*., 2013).
Spirulina and its products can be applied as feed and food additives in agriculture, food industry, medicine, science and cosmetic. It has high contents of macro and micronutrients (Seyede Marzieh Hosseini *et al*., 2013). Several researches for *Spirulina* supplementation into bread have been documented.
According to Cauvain and Young (2009), the addition of a protein source is useful in adjusting the dough when one prepares gluten free bread, preventing the collapse of the product. Thus, in the preparation of gluten free bread using rice flour, *Spirulina* may be indicated to assist in the forming of a network improving the volume of the bread. Shin *et al*. (2010), found a specific bread volume of 1.76 mL.g-1 for rice flour when transglutaminase was added and the specific volume increased to 1.93 mL.g-1 when soy protein isolate was added.

This microalgae powder can be added to improve the nutritional value of bread. Microalgae bread has color and flavor of algae and higher amounts of vitamins, microelements, especially the active biological material. Microalgae improved the water holding in the bread and cause to the long-term shelf time of product (Danesi *et al*. 2010)

Figueira *et al*. (2011) observed that the addition up to 4% of *Spirulina* on rice based flour caused no significant difference in the volume of rice flour bread, when the concentrations of hydrocolloid and transglutaminase were 2.0% and 0.5%, respectively.

There was improvement in the technological characteristics of rice flour bread, using methylcellulose, transglutaminase and *Spirulina*, allowing one to define formulations with good technological, chemical and sensory characteristics. The hydrocolloid methylcellulose was important to improve the characteristics of the specific volume and crumb firmness at levels above 1.5%. The enzyme transglutaminase was effective in promoting the formation of protein network in concentrations from 0.2 % to 0.6 % depending on the amount of *Spirulina*

added. The increase in concentration from 1% to 4% of *Spirulina* in the formulations of rice flour bread did not affect the preference of the judges and increased protein content by 20%, and improving the specific volume and crumb firmness (Selmo, M. S. *et al*., 2014).

Estefanía Rodríguez De Marco *et al*. (2014) evaluate the effect of the incorporation of *Spirulina* on technological and nutritional quality of dried pasta. Wheat flour was substituted by *Spirulina* biomass at three levels: 5, 10 and 20 g/100 g, and a sample without *Spirulina* biomass was made as control. The technological quality was analyzed in terms of cooking properties and texture profile, while pasta surface was observed by confocal microscopy. In addition, protein content, *in vitro* protein digestibility, phenolic compound content and *in vitro* antioxidant activity were the major bio-functional characteristics measured. An *in vitro* starch digestion was performed in order to estimate the glycemic index. Only pasta with 20 g of *Spirulina* / 100 g of flour did slightly modify technological quality parameters; microstructure studies revealed the impact of *Spirulina* addition, resulting in a more heterogeneous surface. The glycemic index was not affected by the addition of *Spirulina*. The incorporation of *Spirulina* resulted in an increase of protein content; however, protein digestibility was reduced as microalgae content increased. Pasta with *Spirulina* exhibited high phenolic compounds content and antioxidant activity compared to control pasta, which could be used to enhance the nutritional profile of the product.

Purpose of our research is to diversify bread products with more nutrients which are similar to functional food. Our research mainly focuses on investigation the effect of yeast ratio, *Spirulina* ratio to bread volume, acidity and other sensory characteristics.

2. Material & Method

2.1 Material

Algae *Spirulina platensis* in powder form is supplied from Chau Dai Duong Co. Ltd in HCM City, Vietnam. Wheat is purchased from Binh Dong Wheatflour Co. Ltd. Yeast saccharomyces cerevisiae comes from Mauri Co. Ltd. Moreover, we also use salt, sugar, butter, egg, milk powder, starch, soybean powder, emulsifier, anti-oxidant, enzyme, potassium sorbate, water.

2.2 Research method

2.2.1 Effect of yeast supplementation (without algae)

Weigh wheat powder, sieve and divide into 7 parts. Prepare ingredient such as salt, water, yeast, additives, sugar, butter, egg, milk powder. Investigate different yeast ratios (0%, 0.25%, 0.5%, 0.75%, 1.00%, 1.25%; 1.50%).

2.2.2 Effect of Spirulina supplementation into breast to human consumption

In order to choose the appropriate algae ratio, we must survey minimum and maximum dose of algae that human can consume in day. We can indirectly survey through bread consumption daily (0g, 100g, 200g, 300g, 400g).

2.2.3 Effect of Spirulina supplementation to bread volume and acidity

Weigh wheat powder, sieve and divide into 10 parts. Prepare ingredient such as salt, water, yeast, additives, sugar, butter, egg, milk powder. Investigate different *Spirulina* algae ratios

2.2.4 Supplemented Spirulina bread nutrition

Analyse bread nutrition which is not supplemented algae (M0) and supplemented algae (M1, M2, M3)

2.2.5 Sensory characteristics of supplemented Spirulina bread

Supplemented *Spirulina* bread should be inspected some sensory characteristics: color, aroma, taste, appearance.

2.2.6 Effect of preservative to the supplemented Spirulina bread shelf-life

Bread has short shelf-life so we investigate effect of potassium sorbate (0.005%, 0.01 and 0.15%) to sensory characteristics and acidity of bread so that we know product shelf-life.

2.3 Statistical analyses

Use Microsoft Excel 2003 at 95% confidence level.

3. Result & Discussion

3.1 Effect of yeast supplementation

Yeast supplementation has affected to bread volume (see figure 1).

The more yeast supplementation is, the more bread volume we get. Sample with 1.25% yeast has the highest bread volume. However it has low elasticity. In case of 1% yeast addition, sample has high volume, good elasticity. If yeast addition is lower than 1%, sample has small volume, non-uniform. So wee choose 1% yeast supplementation (10 g yeast/ kg dry wheat flour) for further experiments.

3.2 Effect of *Spirulina* supplementation into bread for human consumption

Maximum daily intake of *Spirulina* is regulated 2 –3g/day for elder and 1 – 2g/day for children (from research data of Angle Life Co. Ltd, Japan). From this reference, we survey on 100 students about daily bread consumption.

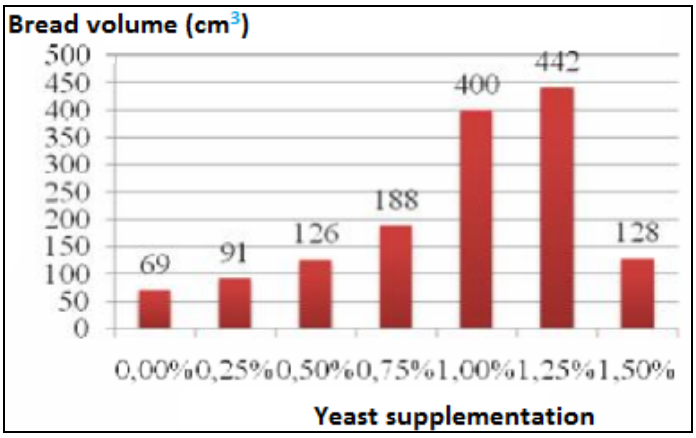

Fig 1: Effect of yeast supplementation to volume of sweet bread

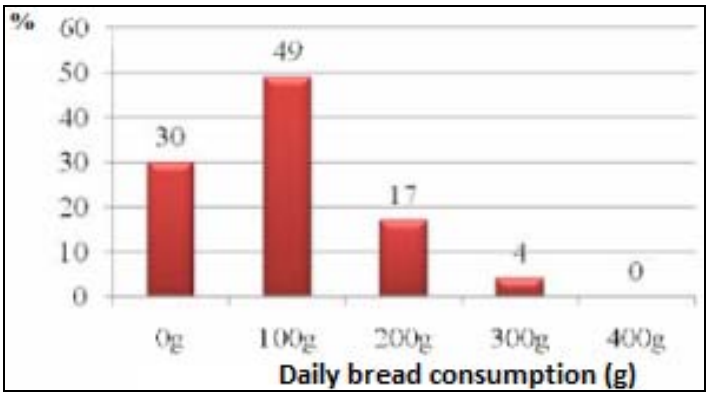

Fig 2: Percentage (%) of person eating bread

From figure 2, we see that consumer group uses 100 g of bread with the highest percentage (49%). This amount is equivalent to 1 – 3g *Spirulina* per day for one person. So in the next experiment we survey the algae ratios 1%, 2% and 3% for sweet bread.

Table 1: Encoded name of bread samples

Code	Description
M0	Control (without *Spirulina*)
M11	1% *Spirulina,* supplemented directly into dough
M12	1% *Spirulina,* pretreated before supplementation into dough (by treating with NaOH 1N and neutralizing with HCl)
M13	1% *Spirulina,* directly supplemented into bread core
M21	2% *Spirulina,* supplemented directly into dough
M22	2% *Spirulina,* pretreated before supplementation into dough (by treating with NaOH 1N and neutralizing with HCl)
M23	2% *Spirulina,* directly supplemented into bread core
M31	3% *Spirulina,* supplemente directly into dough
M32	3% *Spirulina,* pretreated before supplementation into dough (by treating with NaOH 1N and neutralizing with HCl)
M33	3% *Spirulina,* directly supplemented into bread core

Fig 3: Bread M12 and M0 before baking

Fig 4: Bread M11 before and after baking

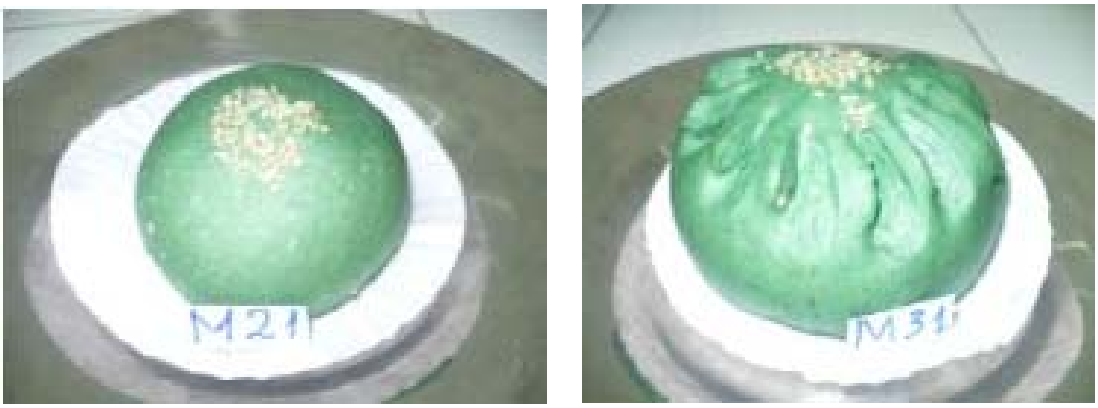

Fig 5: M21 and M31 before baking

Fig 6: Bread M13 after baking

Fig 7: Bread M33 and M23 after baking

Fig 8: M1 before and after baking

Fig 9: M13, M23 and M33 before baking

3.3 Effect of *Spirulina* supplementation to bread quality

3.3.1 Effect of *Spirulina* supplementation to bread volume

Weight of each bread roll before baking is 70 g. After baking, its weight is normally 65 – 67g.

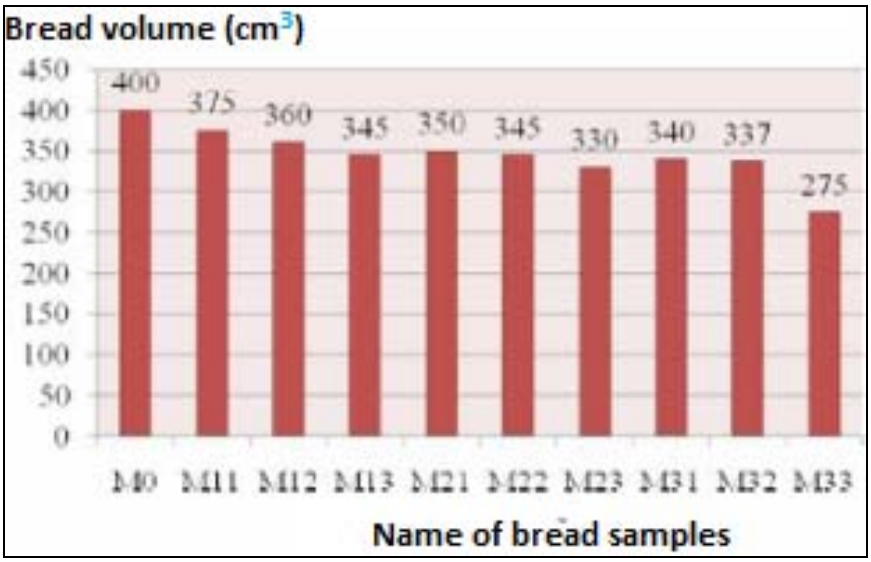

Fig 10: Effect of *Spirulina* supplementation to bread volume

With *Spirulina* supplementation, volume of bread after baking is reduced 6.25% – 31.25%.

3.3.2 Effect of *Spirulina* supplementation to bread acidity

Normal bread has acidity about 1.9 °N. However, supplemented samples have acidity in range 2.3 – 2.6 °N.

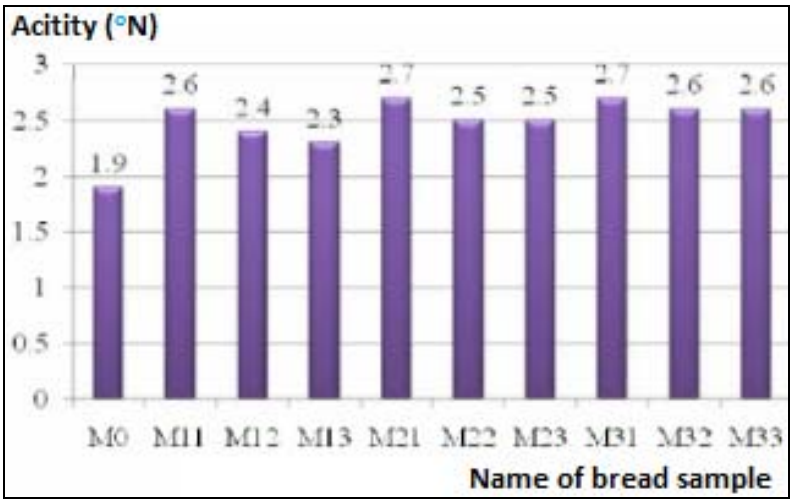

Fig 11: Effect of Spirulina supplementation to bread acidity

Spirulina supplementation into bread creates sour feeling but not significantly different.

3.4. Nutrient compositions in the *Spirulina* supplemented bread

We verify the nutrient compositions in the *Spirulina* supplemented bread after preparation (M0: without *Spirulina,* M1: 1% *Spirulina,* M2: 2% *Spirulina,* M3: 3% *Spirulina).* Results are as follows:

Table 2: Nutrient compositions in the *Spirulina* supplemented bread (based on 100 g)

Description	Unit	M0	M1	M2	M3
Protein	%	9.6	9.9	10.4	11.0
Lipid	%	5.0	5.2	5.6	5.7
Carbohydrate	%	56.0	55.5	54.8	54.2
Moisture	%	28.1	27.6	26.8	26.0
Total mineral	%	1.3	1.8	2.4	3.1
Calcium	mg	60.0	70.0	81.0	90.0
Iron	mg	1.5	1.8	2.3	2.7
Phospho	mg	100.0	108.2	117.1	125.0
Energy	Kcal	307.4	308.4	311.2	312.1

In the table 2 above, nutritional value of the *Spirulina* supplemented bread is enhanced dramatically.

3.5 Sensory characteristics of the *Spirulina* supplemented bread

3.5.1 Color characteristics of the *Spirulina* supplemented bread

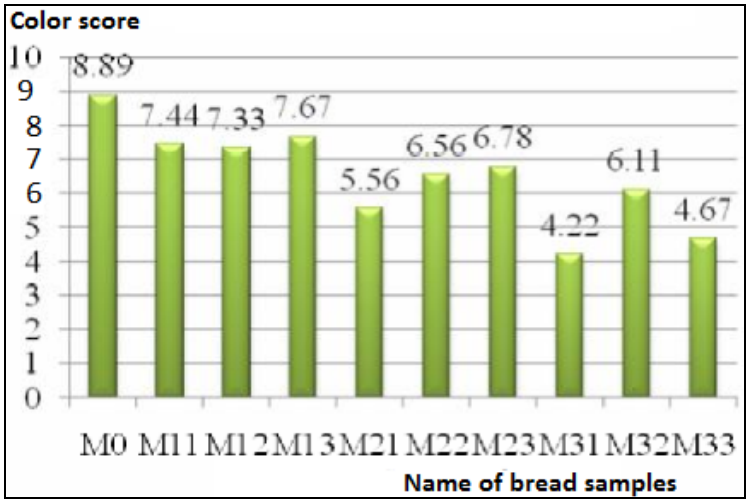

Fig 12: Color score of *Spirulina* supplemented bread.

Sample M13 has the highest color score. Meanwhile sample M31 has the lowest color score.

3.5.2 Aroma characteristics of the *Spirulina* supplemented bread

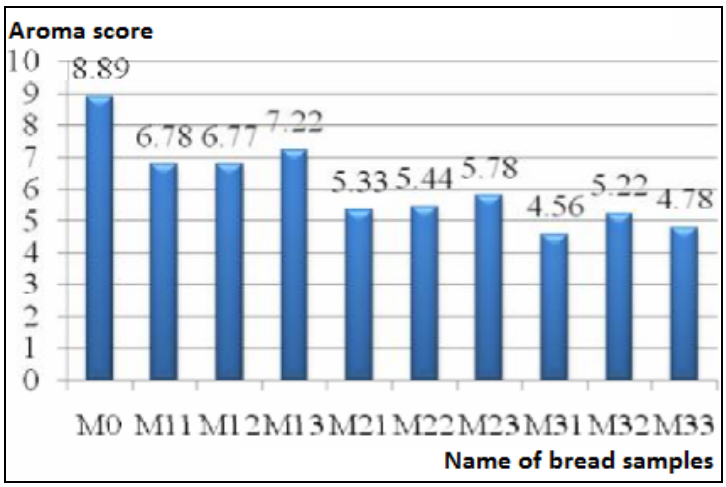

Fig 13: Aroma score of *Spirulina* supplemented bread.
There is significant difference about aroma among supplemented samples, but not significant difference between control sample and supplemented sample.

3.5.3 Taste characteristics of the *Spirulina* supplemented bread

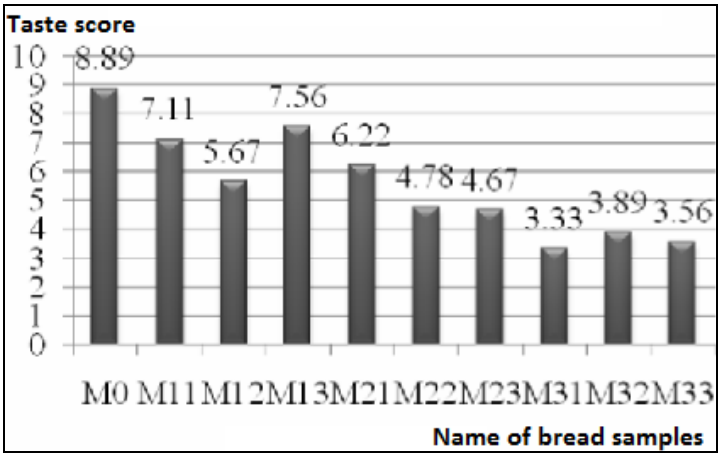

Fig 14: Taste score of *Spirulina* supplemented bread.
From figure 14, there is significant difference regarding to samples supplemented *Spirulina* 1%, 2% and 3%.

3.5.4 Appearance characteristics of the *Spirulina* supplemented bread

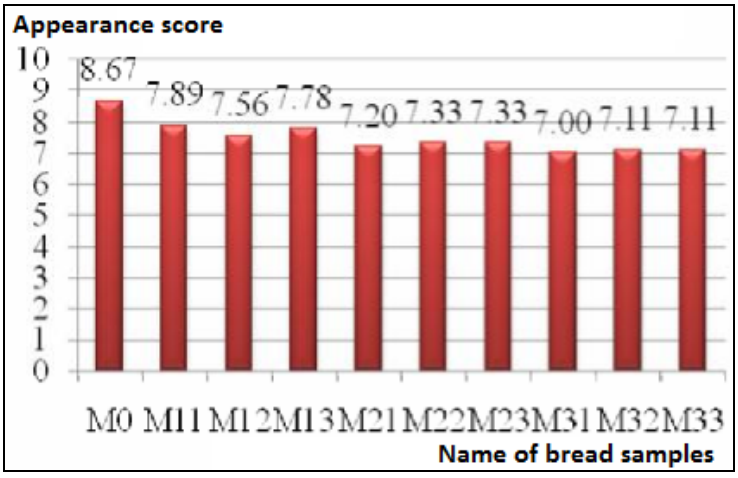

Fig 15: Appearance score of *Spirulina* supplemented bread.
There is significant difference about appearance among supplemented samples, but not significant difference between control sample and supplemented sample.

3.6 Effect of preservative ratio to the *Spirulina* supplemented bread shelf-life

Table 3: Effect of preservative ratio to the *Spirulina* supplemented bread shelf-life

Sample	Shelf-life of bread without potassium ascorbate (days)	Shelf-life of bread 0.05% potassium ascorbate (days)	Shelf-life of bread 0.1% potassium ascorbate (days)	Shelf-life of bread 0.15% potassium ascorbate (days)
M0	3	7	10	12
M11	2	6	9	11
M12	3	6	9	12
M13	3	7	10	12
M21	3	6	9	11
M22	2	6	9	11
M23	3	7	10	12
M31	3	6	9	11
M32	2	6	9	11
M33	3	6	9	11

We decide to choose 0.05% potassium ascorbate to preserve the Spirulina supplemented bread.

3.7 Microrganism in the *Spirulina* supplemented bread

Fig 16: The excellent *Spirulina* (M13) bread

Table 4: Microorganism in the *Spirulina* supplemented bread

No	Criteria	Unit	Result	Standard 867 BYT-1998
1	Yeast	CFU/g	<10	10^2/g
2	Mold	CFU/g	<10	10^4/g
3	TPC	CFU/g	$2.0.10^1$	10/g
4	*Coliform*	CFU/g	<10	10/g
5	*E.coli*	CFU/g	<10	3/g
6	*Bacillus cereus*	MPN/g	<3	10/g

Table 5: Nutritional composition in bread M13

Description	Unit	M13	
Protein	%	9.9	
Lipid	%	5.2	
Carbohydrate	%	55.5	
Moisture	%	27.6	
Total mineral	%	1.8	
Calcium	mg	70.0	
Iron	mg	1.8	
Phospho	mg	108.2	
Energy	Kcal	308.4	

4. Conclusion

Research results show that using *Spirulina* powder in food products is very practical and functional. Increased demand for healthy food, more global acceptance of fortified foods by S. platensis, low production cost and its high nutritional content enhance its application in food industry. This microalgae powder can be added to improve the nutritional value of bread. Microalgae bread has color and flavor of algae and higher amounts of vitamins, microelements, especially the active biological material. Microalgae improved the water holding in the bread and cause to the long-term shelf time of product.

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