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Development of diabetic muffin enriched with flaxseed and its chemical and microbial analyses

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Abstract
A Diabetic patient often has a passion for sweet but by keeping blood glucose level normal. Thus an attempt was made to develop a high fiber Diabetic muffin fortified with flaxseeds and using a sugar substitute in the form of orange pulp and honey. It was followed by sensory evaluation by 20 diabetic panel members and comparative shelf life study to diagnose the better mode of storage and longevity. It was followed by comparative chemical analyses of the standard product and most accepted variation (product) stored at refrigeration and microbial analyses of the most accepted in winter and summer.
It was seen that most acceptable product had lower amount of carbohydrate as compared to the standard product. The ash content is same in both. But it has higher moisture, ash, fat, fiber and mineral (calcium, phosphorus and iron) content than the standard product. The standard and the most acceptable product were more stable on refrigeration than at room temperature. The longevity of the most acceptable product varied with season from nine days in winter to five days in summer. Also there were seasonal variations observed in various microbial counts. Thus it can be marketed as a diabetic friendly baked product at consumer level in future.
Further effective methods of chemical analyses on macronutrients and micronutrients contents are required followed by animal testing and human testing to prove its efficacy as a low glycemic index food and with low glycemic load food in near future.

Keywords: Street foods, Hygiene, Vendors, Food safety

Introduction [13]

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both leading to hyperglycemia with disturbance in carbohydrate, protein and fat metabolism (Kumar *et al*. 2002; Beverley *et al*. 2003; Lindberg *et al*. 2004). The diabetic diet comprises of a high fibre, adequate protein, low fat and moderate carbohydrate of 100g (minimum) carbohydrate per day. Besides optimal amount of minerals like calcium, phosphorus, iron and sodium are essential.
Snacking in general can help spread out food intake over the course of a day, helping to lower blood lipids and glucose for people with type 2 diabetes.
The long term survival of a business depends on its ability to successfully introduce a new product in the market. Regularly establishing new products can potentially provide satisfaction to the continuous changing customers' requirements and demands. [7, 34]
Bakery products and cereals are a valuable source of nutrients in our diet providing us with most of our food calories and approximately half of our protein requirements. The nutrients in bakery products are carbohydrates, proteins, lipids, vitamins and minerals. They are susceptible to microbial contamination and hence their microbial analyses is essential. [31]

Aims and Objectives

The aim of the study was to develop a fibre rich diabetic friendly muffin with a sugar substitute enriched with flaxseed followed by chemical and microbial analyses.
The following were the objectives of the study:-

1. Develop a sugar substitute baked product for diabetes.
2. Estimation of shelf life of the product
3. Check the acceptability of the product.
4. Determine the chemical contents of the sample.
5. Assess the safety of the product throughout the year

Methodology
The methodology involved the following.

Product Development: It involved standardization of the product one based on whole wheat flour (Basic A) and the other based on whole wheat flour and rice flakes (Basic B). This was followed by sensory evaluation. Two variations (Variation B1 and Variation B2) on the most acceptable standard product were done. Each variations were fortified with different quantity of flaxseed. The final variation was made with orange pulp and honey as a sugar substitute. Each method was followed by sensory evaluation by 20 diabetic panel members.

Shelf Life Study: A comparative study of shelf life on storage at room temperature and refrigeration was done by room serial dilution method. The best form of storage was determined and was used for further analyses.
It was followed by chemical analyses of the standard and the most acceptable product and microbial analyses of the best product.

Chemical Analyses

Nutrients	Detecting Methods
Total Carbohydrates	Anthrone Method
Protein	Folin Lowry's Method
Fat	Soxhlet Apparatus
Ash	Drying and Combustion
Crude Fibre	Acid and alcohol washing
Calcium	OCPC Method
Phosphorus	Ammonium Molybdate Method
Iron	Thiocyanate Method

Microbial Analyses: It was conducted in winter and summer of the best (most acceptable) product at the predetermined best mode of storage. It include determination of the moisture, water activity, and pH by individual meter in each case. The growth of following micro-organisms were estimated Staphylococcus aureus, Salmonella, *Escherichia coli*, Fungi and mould. Serial dilution and replica plating methods were used as required. In each case selective nutrient media was used followed by confirmatory tests wherever required.

Statistical Analysis: Two way ANOVA (Analysis of Variance) method was used as a statistical tool to determine the difference in the acceptability of the product.

Results and Discussion
Acceptability rate for the products developed

Table 5.1: The average scores of 20 panel members of the standard products and variations

Characteristics / Developed Products	Appearance	Color	Taste	Texture	Odor	Overall rating
Basic A	6	6	5.6	6.6	6	6.04
Basic B	6.5	6	7.5	7	7.5	6.9
Variation B1	7	8	8	8	8.5	7.9
Variation B2	9	9	9	9	8	8.8

Out of the two standard product developed it was seen that Basic B was more preferred as a standard product as compared to Basic A. Out of the two variations prepared it was seen that Variation B2 was more preferred as compared to Variation B1. Also variation B2 was the most preferred product of all the products developed.

Determination of Shelf Life of the same season at two different storage conditions: The Microbial Growth in (CFU/ml) was determined up to the fifth dilution in both the mode of storage.
Refrigeration proved to be a better mode of storage and provided better stability and acceptability to the product as compared to room temperature storage. Thus further analyses after product development and sensory evaluation were carried out on the refrigerated product.
Note: The microbial count of both the standard and the best product at two different storage conditions were almost equal with a variation of ±2. Since microbial analyses were determined only for the best/ most acceptable product, hence only the microbial count of the best product is considered.

Chemical Analyses
The standard or the basic product (Basic B) and the best product (Variation B2) stored at refrigeration were subjected to various chemical analyses and the results obtained were as follows:

Total Carbohydrate

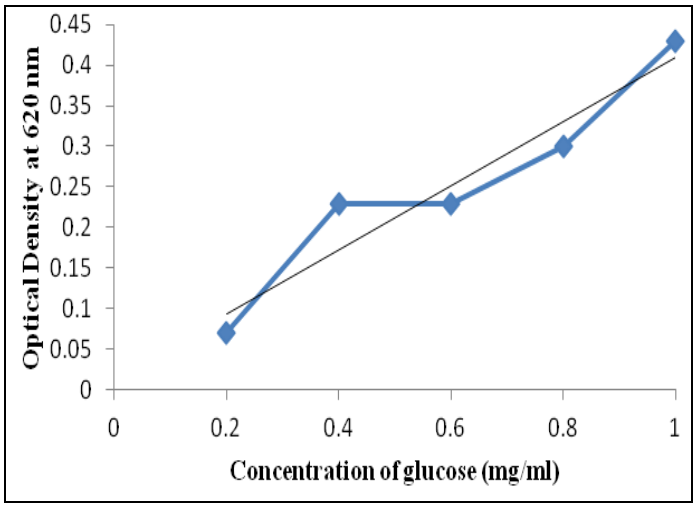

Fig 5.3.1: Standard glucose curve of the considered working standards.

As the total carbohydrate content per 100g of the standard product is 40.97g and 36.075g for the best product/most

acceptable product (Variation B2), the best product can be considered as a better alternative sugar substitute baked item for metabolic disorders like Diabetes mellitus.

Total Protein

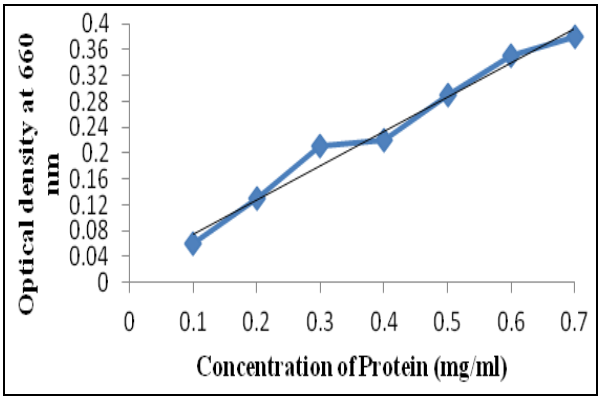

Fig 5.4.1: Standard curve showing protein concentration of the prepared working standards

As carbohydrate contents need to be reduced in Diabetes so adequate amount of calories should come from protein sources. The protein content per 100g of the standard product (Basic B) is 4.88g and the best product (Variation B2) is 5.076g

Fat: Cholesterol in the diet should be upto 200mg/day. This could be achieved by lowering fats of animal origin like butter, ghee, margarine and greater use of fats of plant origin (Vegetable Oil) [33]. The fat content per 100g of the standard product (Basic B) is 36.45g and the best product (Variation B2) is 38g.

Crude Fibre: Crude fibre is a measure of the quantity of indigestible cellulose, pentosans, lignin, and other components of this type in present foods. It refers to the residue of a feed that is insoluble after successive, boiling with dilute acid and alkali. Fibre offers a variety of health benefits and is essential in reducing the risk of chronic disease such as diabetes, obesity, cardiovascular disease and diverticulitis [35]. The crude fiber content per 100g of the standard product (Basic B) is 1.16g and the best product (Variation B2) is 1.26g.

Ash: The ash content per 100g for both the standard (Basic B) and the best products (Variation B2) are 34.19g.

Mineral Contents

Minerals	Products		Significance
	Basic B	Variation B2	
Calcium (mg)	163.41	183.30	It helps in bio synthesis and release of insulin from the Beta cells of the pancreas via exocytosis in glucose metabolism.
Phosphorus (mg)	21.39	23.38	DKA leads to various degree of intracellular phosphate depletion. Excess of it leads to toxicity hampering Calcium absorption.
Iron (mg)	2.08	2.167	Iron Overload increases the risk of DM especially GDM. Iron Deficiency Anemia increases HbA1C level in Diabetic individuals.

Microbial Analyses [25, 27, 34,]

Water activity, pH and high moisture contents are most important factors influencing microbial quality of the product. High moisture containing products and products with high water activity are more likely to present food safety concern. As they support the growth of a wide range of bacteria, yeasts and moulds. However microbial growth also depends upon the places from where the products are made and collected.

The results obtained for various microbial analyses of the most accepted product or the best product (Variation B2) stored at refrigeration were as follows:-

Moisture: The moisture content per 100g in:-Basic B= 39.83g and Variation B2= 57.95g.

Estimation of Seasonal Variation in shelf life: The best accepted refrigerated product was subjected to shelf life testing both in winter (in January) and summer (in June) on Day 0, 3, 5, 7 and 9. The results obtained were as follows.

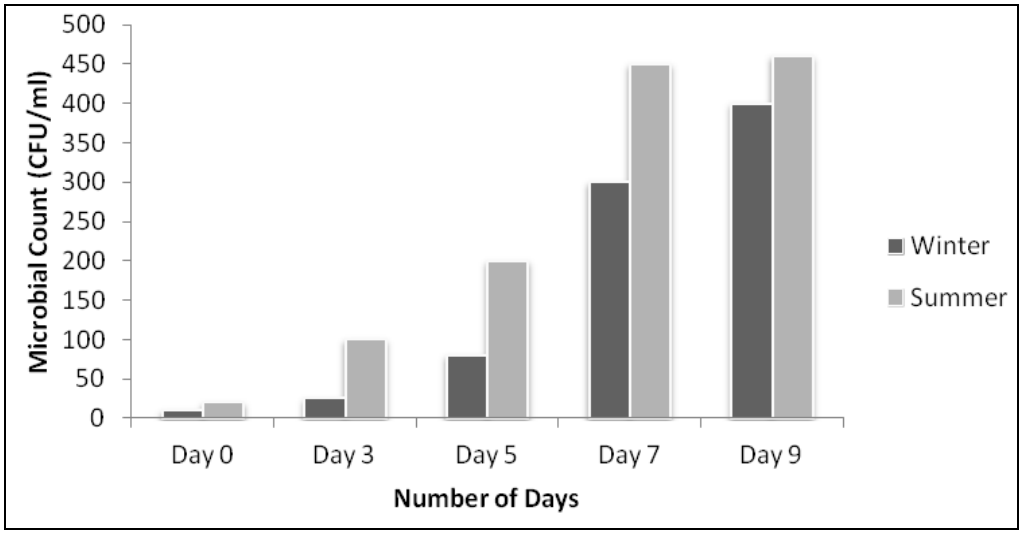

Fig 5.11.1: Seasonal variation in microbial count of the first dilution

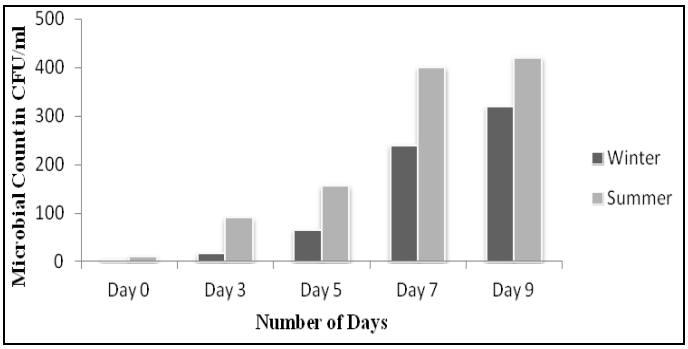

Fig 5.11.2: Seasonal variation in microbial count of the second dilution

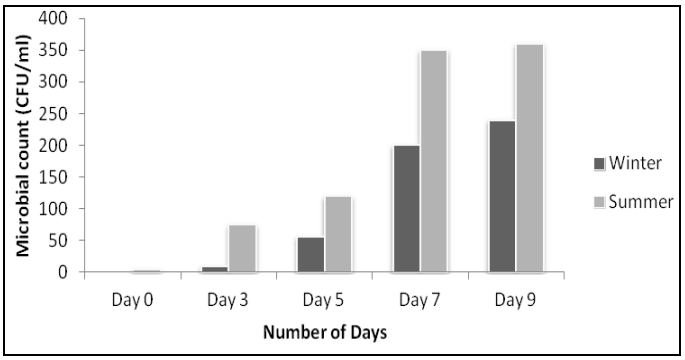

Fig 5.11.3: Seasonal variation in microbial count of the third dilution

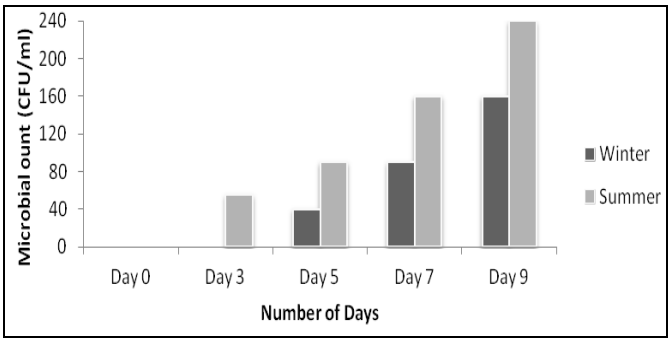

Fig 5.11.4: Seasonal variation in microbial count by serial dilution of the fourth dilution

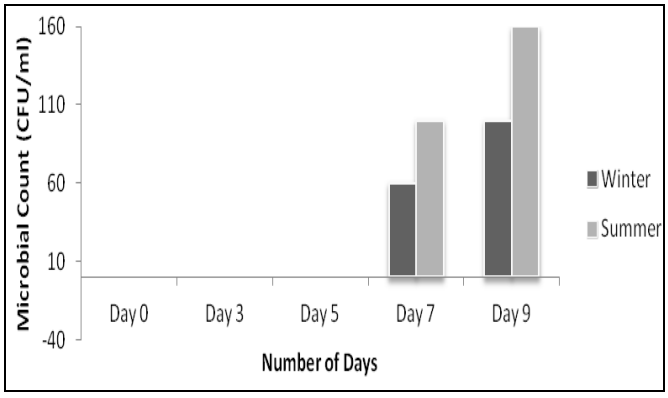

Fig 5.11.5: Seasonal variation in microbial count of the fifth dilution

The desired/acceptable range in bacterial growth colonies is from 30-300 CFU/ml. below this range even if colonies are present, they are considered negligible.

Thus there is a seasonal variation in the growth of microbes in the product. The most acceptable product can be considered safe for consumption upto seventh day from the day of product manufacture in winter and upto fifth day from the day of manufacture in summer months.

Estimation of the Water Activity: The water activity of the best product stored under refrigeration showed a slight increase from Day 0 to day 9 at a room temperature of 27.2 ºC in both the seasons.

Table: 5.4: Water Activity of the best product on respective days

Day 0	Day 3	Day 5	Day 7	Day 9
0.947 a_w	0.950 a_w	0.950 a_w	0.965 a_w	0.969 a_w

Estimation of pH: The pH of the best product stored under refrigeration had a constant pH of 4.8 in all the respective days in both the seasons.

Estimation of Microbes

Staphylococcus aureus (S. aureus): *S. aureus* colonies are gray black to jet black, circular, turbid & moist. Frequently there is a light coloured (off-white) margin, surrounded by opaque zone (precipitate) and frequently with outer clear zone. Colonies have buttery to gummy consistency when touched with the inoculating needle.

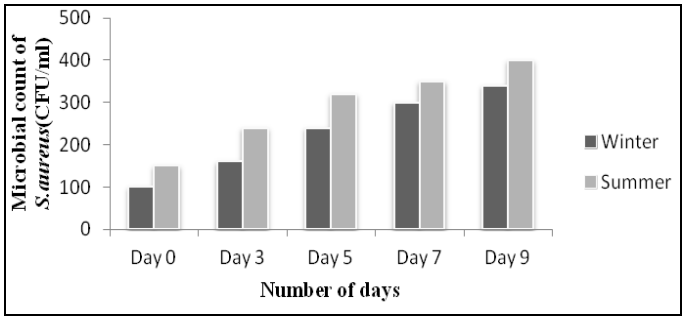

Fig 5.12: Seasonal variation in the microbial count of *Staphylococcus aureus*

Gram staining: Violet colored spherical shaped cells were present in single, paired and in irregular clusters were observed on performing Gram Staining as a confirmatory tests.

Estimation of the growth of Salmonella species: No estimated color change was observed. No growth of the Salmonella species was observed on any of the days in both the season. Thus there was no contamination from raw materials like egg which leads to outbreak of severe diseases.

Estimation of *Escherichia coli:* Purple pink colonies were observed in the Violet Red Bile Lactose (VRBL) Agar in summer. No growth was observed in winter neither by Serial Dilution method nor by Replica Plate Method.

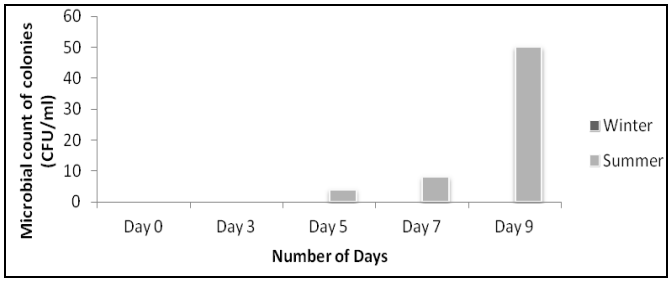

Fig 5.15: Seasonal Variation of the Growth colonies in the VRBL Agar Media

IMVIC Tests

The results of the confirmatory test of both the season of Day0, 5, 7 and 9 were as follow:-

Tests	Winter	Summer
Indole	-	Formation of red colored ring at the top
Methyl Red	-	Development of Red color of the glucose phosphate broth on addition of Methyl Red Reagent
Vogues Proskauer	-	No color change
Simmon Citrate	-	No color change of the slope was observed

Presence of *Escherichia coli* is confirmed on the days growth were observed in summer within acceptable range (4-8 CFU/ml upto 7th day and 50 CFU/ml on ninth day). This may be due to the fact as days passed from the date of manufacture of the product, the product became sticky and released water. Besides there was a marked increase in water activity of the product which is an ideal condition to support microbial growth especially E.coli which is a water-borne pathogen.

Estimation of Fungi and Mould

Serial Dilution Method: There was no visible fungal or mould growth observed in winter. Visible growth was observed in summer months which may be due to post baking contamination. (Knight and Menlove 1961). The count was very less 5-8 CFU/ml in summer. The Reference Range of Fungal Growth within which it is considered acceptable is upto 10 CFU/ml.

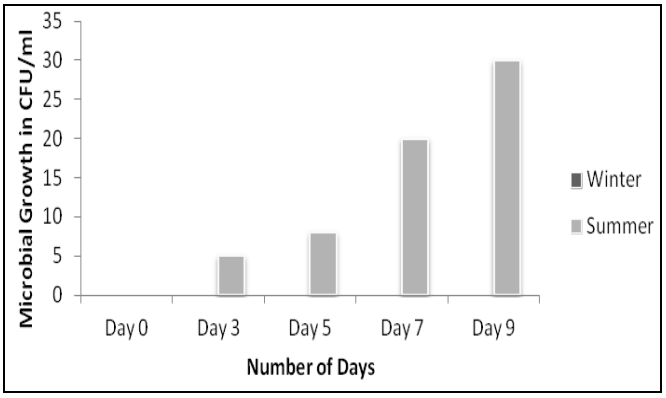

Fig 5.18: Seasonal variation in the fungal and mould growth.

Replica Plating Method

There was no growth of colonies observed in both seasons on any of the days neither in winter nor in summer months.

Statistical Analysis

The tabulated value for F_c at 5% level of significance=5.42. Since the computed T statistic under null hypothesis (H_0) has a lower value compared to tabulated statistic value, hence null hypothesis is accepted at 5% level of significance which implies that there is no significant difference in the average score of the panel members regarding physical parameters (appearance, color, taste, texture, odor and overall).

The tabulated value $F_{5,15}$ at 5% level of significance =4.52. Since computed statistic for the row F_R=28.52 is greater than the tabulated F value at 5% level of significance. Hence H_0 (null hypothesis) is rejected which implies that there is a significant difference in the average score of the panel members regarding the various products (Basic A, Basic B, Variation A_1 and Variation B_2) developed.

Limitations & Future Recommendations

Further effective methods of chemical analyses on macronutrients and micronutrients contents are required followed by animal testing and human testing to prove its efficacy as a low glycemic index (GI) food and with low glycemic load (GL) food in near future.

Conclusion

An attempt was made to develop a diabetic friendly muffin fortified with flaxseed followed by chemical and microbial analyses. There was significant difference in acceptability of products both physically and statistically. No significant difference was observed in physical parameters of the products statistically in sensory evaluation. Refrigeration proved to be a better mode of products' storage. The shelf life of the Best Product (Variation B2) is 9 days in winter and 5 days in summer. It was seen that lower amount of carbohydrate, equal ash content and higher amount of moisture, fat, fibre and minerals contents in the Best product (Variation B2) as compared to the standard product (Basic B).

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