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Kulsum Khan
Department of Food Science &
Nutrition Management, J.D.
Birla Institute, Kolkata

Sweata Rani Rai
Assistant Professor, Department
of Food Science & Nutrition
Management, J.D. Birla
Institute, Kolkata

A study on the acceptability of functional whole wheat bread fortified with mushroom (*Agaricus bisporus*) powder and whey protein isolates amongst gym goers (23-27 years)

Kulsum Khan, Sweata Rani Rai

Abstract

Background: Whey protein isolates has been in strong demand with the gym goers for supporting bone density, building muscles, boosting the digestibility of certain foods and maintaining the integrity of immune system. Moreover, the consumption pattern of bread has been phenomenal in today's era. Keeping in view unique chemical composition of whey protein isolates and mushroom, it was incorporated in daily food items.

Method: A market survey was conducted on gym goers to know their consumption pattern of bread. A protein rich bread was prepared with graded levels of sun-dried mushroom powder (2.5, 5 & 7.5%) and whey protein isolates (2.5 & 5%) by replacing whole wheat flour. Effect of two ingredients incorporation on the nutritional composition and sensory qualities were evaluated. The bread with more consumer acceptability was analyzed for physical and chemical parameters like protein, vitamin-D, calcium, phosphorus, fat and was compared with standard bread. The microbial status of the bread was investigated and interpreted in terms of shelf-life.

Result & Discussion: By considering nutritional and consumer acceptability the bread with 7.5% mushroom powder and 5% whey protein isolates was the best sample. The fortification using whey protein isolates and sun-dried mushroom powder improved the nutritional composition of the bread in terms of better amino acid profile and calcium and Vitamin-D status. These nutrients are of greater significance for muscle development in the case of gym goers.

Conclusion: The functional bread is effective in terms of nutritional quality as well as texture and shelf life.

Keywords: Whey protein isolates (WPI), Sundried mushroom powder, HPLC (High Performance Liquid Chromatography), Gym goers, Vitamin-D, Fortified whole wheat bread.

Introduction

Food fortification has been defined as the addition of one or more nutrients to a food to improve its quality for the people who consume it, usually with the goal of reducing or controlling nutrient deficiency [Mannar, 2010] [9]. Breads could be used as vehicle in the formulation of varying products with the increasing consumer demands for healthful food products. The enrichment of bread and other cereal based confections with milk proteins particularly in regions where people are consuming protein supplements. The whole wheat flour has been shown by many researchers to be a rich source of these functional ingredients such as fibre, phytochemicals, minerals, essential amino acids that are located in the bran and fat soluble vitamins contained in the germ of the whole wheat grain [Dewettinck *et al.*, 2008] [3]. The consumption of large amounts of protein by athletes and bodybuilders is not a new practice. [Willoughby *et al.*, 2007] [17] Normal bread does not contain enough protein for human nutrition. Fortifying bread using two main ingredients sundried mushroom powders and Whey protein isolates is a very effective step to increase protein and other nutrient content in wheat bread. Taste and flavor of mushroom and whey protein isolates can also increase sensory quality of breads. Protein rich bread can be a choice for gym goers due to their need for the consumption of high amount of protein for muscle gain.

Mushrooms can be termed as protein substitutes depending on the variety mushrooms contain 2-4% protein and all the essential amino acids making their protein complete. Mushroom, a Vitamin D source can make a complete protein among the vegetarian category and could be ideal substitute to meat [Koyyalamudi *et al.*, 2009] [6].

Correspondence
Sweata Rani Rai
Assistant Professor, Department
of Food Science & Nutrition
Management, J.D. Birla
Institute, Kolkata

Whey protein is the one which is most useful for sports nutrition. A typical WPI powder will contain approximately 92.0% protein, 4.5% moisture, 2.0% ash, 1.0% fat and 0.5% lactose [Kuntz *et al.*, 2010] ^[7]. Whey proteins have all the essential amino acids and in higher concentrations compared to various vegetable protein sources such as soy, corn, and wheat gluten. In addition to having a full spectrum of amino acids, the amino acids found in whey are efficiently absorbed and utilized, relative to free amino acid solutions. [Marshall, 2004] ^[10]. Present study hence, deals with formulation and development of functional bread with mushroom powder (sundried) and whey protein isolates and to evaluate the products nutritional, microbiological (shelf life), sensory quality and consumer overall acceptability.

2. Materials & Methodology

2.1 Study Design

A market survey was conducted to know the consumption pattern of bread, mushroom and supplements using a questionnaire and the respondents were a total number of 50 members who were gym goers selected from two gyms (23-27 yrs). A fortified protein rich bread was prepared using sundried mushroom powder and whey protein isolates.

Table 1: Formulations of the fortificants used for product development

Variations Ingredients	B (gm/ml)	C (gm/ml)	D (gm/ml)	E (gm/ml)	F (gm/ml)
Whole wheat flour	97.5	95	92.5	90	87.5
Mushroom powder	2.5	5	7.5	7.5	7.5
Whey protein isolates	-	-	-	2.5	5
Yeast	1.5	1.5	1.5	1.5	1.5
Salt	1.5	1.5	1.5	1.5	1.5
Water	60	60	60	60	60
Sugar	6	6	6	6	6
Vegetable fat	4	4	4	4	4

2.3 Sensory evaluation

Sensory evaluation of the fortified bread samples were carried out by 10 panelists on a 9 point hedonic scale for different parameters such as colour, appearance, odour, taste, texture and overall acceptability as described by Joel, 2004. Another sensory evaluation was done to check the acceptability of the fortified bread amongst gym goers.

2.4 Chemical and nutrient analysis

The newly fortified bread was used for the physical, chemical analysis and microbial inspection. Various parameters like moisture, ash, minerals, Vitamin-D, Qualitative determination of amino acid, protein, fat, carbohydrate and crude-fibre, were determined using AOAC (2006) method. Moisture was determined by hot air oven method. Protein estimation was done using Lowry's method; Vitamin-D was analyzed using HPLC (High Performance Liquid Chromatography). Minerals like Calcium and Phosphorus composition was determined by colorimetry.

2.5 Microbiological analysis

The bread was examined for microorganism using total plate count by pour plate technique and by serial dilution method.

2.6 Statistical analysis

The results were analyzed by using student's test for paired difference. Statistical data was conducted to evaluate the acceptability of the fortified bread.

3. Results

The market survey reported that brown bread was being consumed more than white bread ($P < 0.5$ @ 1% level of

White Button Mushroom (*Agaricus bisporus*) was collected from the local Beck Bagan Market, Kolkata. The packet consisted of 200 gms mushroom and costed Rs. 35 per packet. Mushrooms were cut into thin slices and spread in trays, dried for 2 days from morning 7 am to 12 pm. After cooling to room temperature the dried mushrooms were ground into powder in a grinder. Then the powder was sieved and packaged in polythene bags and stored at room temperature for further use in the preparation of bread. Whey protein isolates was bought from the market which costed Rs. 1900 per kg.

2.2 Product development

The whole wheat flour was mixed, with varying inclusions of 2.5, 5, 7.5% of the sun-dried mushroom powder and whey protein isolates 2.5 & 5%. These fortificants were blended with other baking ingredients (Table 1) in a bowl, kneaded for 10 minutes into consistent dough and was proofed twice (1st = 30-45 mins, 2nd = 10-15 mins) the resulting dough was molded and placed in a pre-oiled baking bowl and baked in an oven for 20 mins at 180-200°C. Standard bread made with 100% whole wheat flour was kept as sample A.

significance), and gym goers depended on protein supplements for muscle gain and body building. Supplements like whey protein were being used widely. The experimental results on various chemical and nutritional characteristics of standard and fortified bread are tabulated in table 2. The supplementation of whole wheat flour with the fortificants greatly affected the physico-chemical quality of the fortified bread. There was a significant improvement in the levels of protein, vitamin D, calcium and phosphorous of the fortified bread as compared to the standard bread.

Table 2: Nutritional analysis of the standard and fortified bread

Parameters	Standard bread	Fortified bread
Protein	8.42 gm	14.57 gm
Fat	3.3 gm	4.5 gm
Carbohydrate	72 gm	65 gm
Moisture	28.1%	33.9%
Ash	0.8 gm	1.82 gm
Crude fibre	1.8 gm	2.7 gm
Calcium	135.46 mg	399.49 mg
Phosphorus	27.62 mg	45.05 mg

4. Discussion

In the present study, the ash content of the fortified bread was increased to 1.82gm as compared to 0.8gm in the standard bread. With the incorporation of mushroom powder and whey protein isolates in the bread, the amount of ash content nearly doubled the amount present in standard bread.

The protein content as shown in Figure 1 of the bread was 8.42 gm/100 gm for standard bread and 14.57 gm/100 gm for fortified bread. The increase is as a result of substitution of whole wheat flour with mushroom powder containing 2-4%

protein and whey protein isolates containing 92% protein [Koyyalamudi *et al.*, 2009; Kuntz *et al.*, 2010] [6, 7]. Whey protein is a complete protein and it contains all 20 amino acids and all 9 essential amino acids in amounts proportional to the human body's need which makes it a very useful nutrient for those who wish to obtain more of their protein from non-meat sources [Bos *et al.*, 2006] [2].

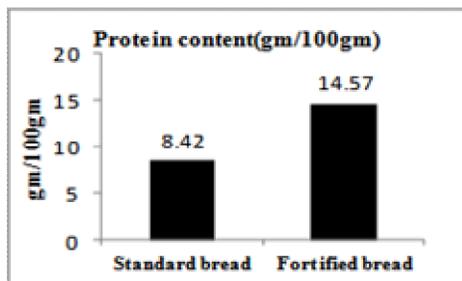


Fig 1: Comparative analysis of protein content

In the present study, the carbohydrate content in fortified bread (65gm/100gm) was reduced in comparison to the standard bread (72gm/100gm). Similar trends were reported by (Mahamud *et al.*, 2012) [8] in the fortification of bread with mushroom powder. Sun-dried mushroom powder and Fortified bread (containing sundried mushroom powder), both were analyzed for their vitamin D content. The amount of Vitamin D was analysed with HPLC method. The result showed that the 5-6 hours exposed mushroom contained 20 microgram or 800 IU / 100 gm, whereas the fortified bread contains 400 IU or 10 microgram of Vitamin D in it. The values vary from country to country because of factors like sunlight, temperature and climate [Philips, 2010] [14].

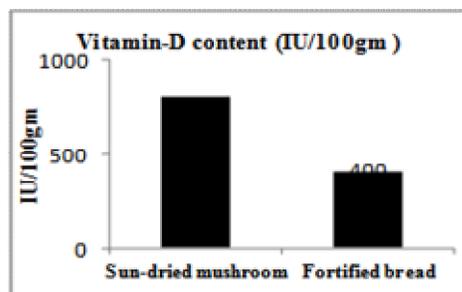


Fig 2: Comparative analysis of vitamin-D content

The crude fibre content of standard bread was 1.8 gm/100gm and fortified bread it was 2.7 gm/100gm. This increase can be due to the incorporation of whey protein isolates and mushroom powder because they are a good source of crude fibre. Similar reports suggest that incorporation of mushrooms in bread and whey protein in yoghurt increased the crude fibre content [Okafor *et al.*, 2012; Guggisberg *et al.*, 2007] [12, 4]. The calcium content as shown in table 2, increased with the incorporation of the fortificants. Increase in calcium content can be due to the mushroom powder and whey protein isolates because both are a rich source of calcium. [Olfati *et al.*, 2009] reported that white button mushroom contains about 3.7-8.4 gm/kg of calcium. Previous researches on whey protein isolates reported a calcium content of 120 mg/30 gm of powder. The results obtained in this study for phosphorus content showed, the standard bread contains 27.62 mg/100 gm and fortified bread contains 45.05 mg/100 gm of the sample. It can be noted that the phosphorus content increased in the fortified product, this can be due to the addition of fortificants as they are a rich source of phosphorus.

Results of the qualitative determination of amino acids (Table 3) showed that the fortified bread contains more amounts of histidine, tryptophan, tyrosine, arginine and methionine, than the standard bread which was proved from the intensity of color, the color was more darker in case of fortified bread. But for amino acid cysteine, results showed that cysteine was present in fortified bread and not in standard bread. A study done by [Walzem & Marshall, 2002 & 2004] [16] confirms that the sulphur-containing amino acids cysteine and methionine are found in high concentrations in whey protein, contributing to enhanced immune function through intracellular conversion to glutathione.

Table 3: Results of the qualitative analysis of amino acid

Amino acid ↓	Standard bread	Fortified bread
Histidine, Tryptophan & Tyrosine	Orange	Red
Arginine and Methionine	Orange	Red
Cysteine	No precipitate	Black precipitate

Sensory evaluation: The result of sensory evaluation of bread samples containing different level of mushroom powder and whey protein isolates substitution as compared to the control is shown in Table 4.

Table 4: Hedonic scale score of the bread (Variations A-F)

Bread type	Appearance	Colour	Taste	Texture	Odour	Overall acceptability
Standard (A)	9	9	9	9	9	9
Variation 1 (B) 2.5% Mushroom powder	7.4	7.3	6.7	6.9	7.2	6.8
Variation 2 (C) 5% Mushroom powder	7.4	7.8	7.6	7.6	7.6	7.6
Variation 3 (D) 7.5% Mushroom powder	8.3	8.3	8.4	8.3	8.3	8.4
Variation 4 (E) 7.5% Mushroom powder & 2.5% whey protein isolates	6.7	6.9	6.4	6.4	6.3	6.5
Variation 5 (F) 7.5% Mushroom powder & 5% whey protein isolates	7.9	7.9	8.0	8.1	7.9	8.1

After the evaluation of the bread, Sample F (with 7.5% mushroom powder and 5% whey protein isolates) was selected to be the best of all the breads and was also acceptable amongst the panel members. After the selection of the final product, sensory evaluation was done by gym goers to check the acceptability amongst them. Rating was acceptable on the

range of 8 as per the hedonic scale. 78% of the respondents agreed that fortification was beneficial. ($p < 0.5$ @ 1% level of significance), indicating that more than half of the respondents preferred the newly fortified brown bread.

The microbial analysis showed (table 5 and 6) that as the number of days increased the colony forming units increased.

Thus the bread should be consumed within 3 days when kept under room temperature whereas upon refrigerated storage the shelf life increased to 5 days. This can be due to the suppression of micro-organisms at refrigerated storage.

Table 5: Microbial count of fortified bread at room temperature

Number of days	cfu/ml $\times 10^3$
Day 3	0
Day 4	27
Day 5	60

Table 6: Microbial count of fortified bread at refrigerated temperature

Number of days	cfu/ml $\times 10^3$
Day 6	15
Day 7	43
Day 8	92

Conclusion: The results showed that newly fortified bread has better nutritional composition compared to the standard bread. Improved protein profile with respect to amino acids can be contributed to the incorporation of whey protein isolates. Vitamin D which is completely absent in standard bread and made available in the fortified bread due to the addition of sun-dried mushroom contributes to bones and muscle development for the gym goers. Furthermore, improvement was also seen in the levels of calcium and phosphorus in the fortified bread beneficial to the gym goers for better body building and improved muscle mass.

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