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# Preparation and evaluation of high protein biscuits containing whey protein concentrate

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Biscuits prepared from the blends containing 0, 10, 20 and 30% of whey protein concentrate (WPC) were evaluated for nutritional, textural and sensory characteristics. The protein and ash contents of WPC containing biscuits were significantly higher than the control ($p<0.05$). The protein content of control sample was 5.2%, while it was 14.1% in the 30% WPC containing biscuits. Blending of refined wheat flour with WPC upto 30% did not have any adverse effect on the sensory quality of protein-enriched biscuits. The cutting and compression strengths of the 30% WPC incorporated biscuits were significantly higher than the control. These protein-enriched biscuits can be stored for 60 days at ambient temperature (30–35$^0$C).

**Keywords:** Biscuits, Whey protein concentrate, Protein, Quality

Bakery products are increasingly becoming popular in India as indicated by over 2.5 fold increase in their production during the last 2 decades (Puranik 2003). India produces 3 million tonnes of bakery products, which can be categorized into bread, biscuits, and cake (Samanth 2002). Biscuit along with bread form major baked food produced in India accounting for over 30 and 50% of total bakery products, respectively (Shukla et al 2000). The per capita consumption of biscuit in India is reported to be 8 kg per annum as against 15 kg per annum in developed countries (Shukla et al 2000).

Protein fortification of food is of current interest because of government guidelines and policies across the globe to combat protein energy malnutrition. The protein-fortified biscuits contain nutrients in concentrated form useful for feeding programs at institutes such as daycare centres and schools or for emergency rations (Tyagi et al 2007). These biscuits can be prepared from composite flours such as wheat flour fortified with soy, cottonseed, peanut, mustard or corn germ flour (Tsen 1976) and also from vital wheat gluten and milk powder (Manley 2001). Dairy ingredients are preferred ingredients due to their functional supremacy and good flavour, colour and nutritional profile (Gupta 2006). Whey protein concentrate (WPC) are dairy ingredients that are highly nutritious, with a protein efficiency ratio of 3.6 and a protein digestibility corrected amino acid score of 1.14 as against 1.5 and 0.25 for wheat proteins, respectively (Pasin and Miller 2004). Besides containing significant amounts of essential amino acids, whey proteins possess excellent biologically active peptide sequences that promote good health (Pihlanto and Korhonen 2003).

Wheat flour has a protein content of 12 – 16% and is very much deficient in lysine and other essential amino acids (Belitz and Grosch 1999). Being a high quality protein with significant quantities of essential amino acids such as lysine, methionine and tryptophan, WPC can be used for supplementing plant proteins in developing high protein foods and other special dietetic foods (Renner 1992). Earlier work on the development of protein-enriched biscuits involved addition of mustard protein concentrate and cottonseed protein isolate (Rajput et al 1988), mustard flour (Tyagi et al 2007), sorghum (Rao and Shurpalekar 1976, Rao et al 1984), soybean (Singh et al 2000, Gandhi et al 2001), fenugreek flour (Hooda and Jood 2005), skim milk powder (Rao et al 1984), sodium caseinate (Bassi and De 1973, Gallagher et al 2005) and WPC (Gallagher et al 2005). The published information on the development of high protein biscuits using WPC is scanty. Hence the present work was undertaken.

## Materials and methods

WPC was procured from M/s Mahaan Proteins Limited, New Delhi. The composition (AOAC 1984) and quality attributes of WPC are given in Table 1. Other materials like refined wheat flour (*maida*), sugar, hydrogenated vegetable oil, leavening agents, skimmed milk powder and salt were procured from the local supermarket.

*Product development:* Biscuit blends from refined wheat flour and WPC were prepared in ratios of 100:0, 90:10, 80:20 and 70:30. Biscuits were prepared using the creaming method outlined by Shalini and Devi (2007), with slight modifications in the recipe during preliminary trials. The modifications included addition of ammonium bicarbonate, lecithin, and vanilla essence. The standardized recipe of the biscuit had the ingredients such as 100 g refined wheat flour, 45 g powdered sugar, 35 g hydrogenated vegetable oil, 1 g salt, 0.4 g baking powder, 0.85 g ammonium bicarbonate, 0.35 g soy lecithin,

Table 1. Quality of whey protein concentrate

| | |
|---|---|
| Colour | Creamy white |
| Taste | Bland |
| pH | 6.4 |
| Bulk density g/ml | 0.42 |
| Insolubility index, ml | 1.6 |
| Moisture, % | 3.8 |
| Fat, % | 5.0 |
| Protein, % (on dry basis) | 70.0 |
| Total minerals, % | 4.5 |
| Total plate count, per g | 500 |
| Coliforms, per 0.1 g | ND |
| *Salmonella,* per 100 g | ND |
| Yeast and mold count, per g | 10 |

ND: Not Detected (n=3)

0.5 ml vanilla essence, and required amount of water. Hydrogenated vegetable oil and ground sugar were taken and rubbed to a creamy consistency. Wheat flour and baking powder were sifted and added to the above mixture and folded in by hand till crumbly texture was reached. Measured quantity of water was divided into 2 approximate parts. In one part, salt was dissolved and in the other ammonium bicarbonate was dissolved. Both were then added to the above mixture and kneaded to smooth homogenous mass along with vanilla essence. The dough was rolled out into thin sheets of 3 - 5 mm thickness by means of a wooden roller. The sheets were cut into desired shape by means of a biscuit – cutter mould. The pieces were placed over wire gauze trays of oven in single layers and baked at $240 \pm 10^0$C for 6 min. The baked biscuits were removed from the trays and cooled to room temperature.

*Sensroy evaluation:* Sensory quality characteristics were evaluated by an experienced panel of 10 members of the laboratory using a 5-point Hedonic scale (Ranganna 1986). The panelists evaluated the products for their colour and appearance, texture, taste, flavour, and overall acceptability. The texture of the products was assessed in a Instron universal testing machine (Shimadzu E2 food tester, model SM – 500 N - 168) for compression and cutting strength, using a knife-edge jig blade set and 50 kg load cell. The samples were halved and placed centrally under the knife-edge.

Biscuit samples were analyzed for proximate composition (AOAC 1984) and acid insoluble ash (ISI 1989). The carbohydrate content of the samples was calculated by difference. The calorie content of the products was computed by the method reported by Gopalan et al (1989).

*Storage studies*: Control and experimental biscuits were packed and sealed in metallized polypropylene pouches having a thickness of 40 µm and stored at room temperature (30-35$^0$C) for 60 days. The sensory quality of biscuits was evaluated at fresh (0 day) and after 30 and 60 days from the date of preparation using the score card described above. The products were also analyzed periodically for moisture and acidity of the extracted fat (ISI 1989). The microbiological quality of biscuits was assessed by determining standard plate counts (SPC) and yeast and mold counts (Harrigan 1998).

*Statistical analysis*: The experiments were carried out in 3 replicates. The results of sensory quality, moisture and acidity were statistically analyzed on a completely randomized block design (CRBD) using the analysis of variance (ANOVA) technique (Snedecor and Cochran 1989).

## Results and discussion

With the increase in the incorporation of WPC in the refined wheat flour, there was an increase in protein and total ash contents and decrease in fat, carbohydrate and crude fiber (Table 2). The protein content increased from 5.2 to 14.1% with increasing the WPC in the blend from 0 to 30%. The protein content in the WPC blended biscuits was significantly higher than that of control ($p<0.05$). The increase in the protein content of WPC supplemented biscuits might be due to the appreciably higher protein content of WPC. Visalakshi and Mohanasundari (2002) also reported higher protein content in WPC incorporated *ghee* and cheese biscuits. Similarly, Bassi and De (1973) reported that protein content of HPB made by using sodium caseinate was 16.6%. The biscuit made by incorporating 30% WPC had protein percentage more than the minimum specified for HPB, i.e. 12% (ISI 1986). The ash content in the 30% WPC incorporated biscuits was significantly higher than the control ($p<0.05$) (Table 2). The ash content of WPC is reported to be between 2.5 and 6% (Morr and Ha 1993). The increase in ash content is obviously due to higher ash content of WPC than refined wheat flour. Narain and Subashini (2004) reported similar observation of higher ash content in the WPC incorporated bread. There was no change in the acid insoluble ash content of WPC blended biscuits.

With the increase in the level of WPC, cutting strength and compression strength values of biscuits increased (Table 2). The cutting strength of biscuits with 20 and 30% WPC was significantly higher than the control ($p<0.05$). Gallagher et al (2005) reported similar observations while studying the effect of different dairy protein powders on biscuits. Similar observations were also made by Tyagi et al (2007), who reported significant increase in the cutting strength values with the increased level of mustard flour incorporation into biscuits ($p<0.05$). The compression strength of biscuits with 30% WPC was significantly higher than the control ($p<0.05$). The high values indicate that the WPC blended biscuits were harder than the control. This could be due to high gelling ability of milk proteins, holding recipe water, fat and other ingredients in a tight matrix, resulting in firmer biscuits (Gallagher et al 2005).

The sensory scores for the colour and appearance increased while flavour, texture and overall acceptability decreased for the biscuits with the increase in the levels of WPC incorporation (Table 3). The improved scores for colour and appearance could be due to the Maillard

Table 2. Quality of WPC incorporated biscuits

| | WPC incorporation, % | | | | CD at 5% |
|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | |
| Moisture, % | 2.7 | 2.8 | 2.8 | 2.9 | 0.65 |
| Fat, % | 20.5 | 20.0 | 19.2 | 18.5 | 1.53 |
| Protein, % | 5.2 | 8.1 | 11.7 | 14.1 | 0.36 |
| Carbohydrates, % | 70.0 | 67.5 | 64.4 | 62.6 | 1.72 |
| Crude fiber, % | 0.5 | 0.4 | 0.4 | 0.4 | 0.02 |
| Ash, % | 1.1 | 1.3 | 1.4 | 1.5 | 0.07 |
| Acid insoluble ash, % | 0.2 | 0.2 | 0.2 | 0.2 | 0.03 |
| Energy, kcal | 486 | 482 | 477 | 473 | |
| **Instron texture** | | | | | |
| Cutting strength, N | 21.4 | 21.6 | 33.5 | 33.8 | 1.57 |
| Compression strength, N (n=3) | 15.3 | 15.4 | 15.6 | 16.3 | 0.91 |

Table 3. Changes in chemical, microbiological and sensory quality of biscuits containing different levels of WPC during storage

| | Levels of WPC incorporation, % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | 10 | | | 20 | | | 30 | | |
| | 0d | 30d | 60d | 0d | 30d | 60d | 0d | 30d | 60d | 0d | 30d | 60d |
| **Chemical** | | | | | | | | | | | | |
| Moisture, % | 2.7 | 2.7 | 2.9 | 2.8 | 2.8 | 2.9 | 2.8 | 2.9 | 3.0 | 2.9 | 2.9 | 3.1 |
| Acidity, % | 1.1 | 1.1 | 1.2 | 1.1 | 1.1 | 1.2 | 1.1 | 1.1 | 1.2 | 1.1 | 1.1 | 1.2 |
| **Microbiological** | | | | | | | | | | | | |
| TVB, cfu/g | ND | 10 | 20 | ND | 20 | 35 | ND | 40 | 45 | ND | 30 | 35 |
| YMC cfu/g | ND | 10 | 40 | ND | 10 | 25 | ND | 20 | 25 | ND | 10 | 20 |
| **Sensory scores** | | | | | | | | | | | | |
| Colour and appearance | 4.1 | 4.0 | 3.9 | 4.1 | 3.8 | 3.7 | 4.2 | 4.3 | 4.0 | 4.3 | 3.9 | 3.9 |
| Texture | 4.8 | 3.8 | 3.8 | 4.6 | 4.1 | 3.8 | 4.5 | 4.2 | 4.1 | 4.3 | 4.3 | 4.0 |
| Taste | 4.5 | 4.4 | 4.4 | 4.5 | 4.4 | 4.1 | 4.6 | 4.1 | 4.1 | 4.5 | 4.1 | 4.1 |
| Flavour | 4.9 | 4.6 | 4.1 | 4.6 | 4.7 | 4.2 | 4.8 | 4.5 | 4.4 | 4.5 | 4.2 | 4.0 |
| Overall quality | 4.6 | 4.2 | 3.9 | 4.5 | 4.3 | 3.9 | 4.4 | 4.3 | 4.0 | 4.2 | 4.0 | 4.0 |

ND: Not detected, d: Storage period in days, TVB: Total viable bacteria, YMC: Yeast and mold count,
Max. sensory scores: 5 – Excellent, 4 – Very good, 3 – Good, 2 – Fair, 1 – Poor, n=3

reaction. Similar observations were made by Bassi and De (1973) who developed high protein biscuit using sodium caseinate. The low scores for the flavour of biscuits incorporated with WPC could be due to its flavour binding capacity. Thapa and Gupta (1996) reported that higher levels of WPC addition imparted milder flavour to the processed cheese foods. While Jayaprakasha et al (2000) observed that flavour scores decreased in frozen yogurt when the substitution of skim milk solids increased from 10 to 50%. However, there was no significant difference in overall acceptability of biscuits upto 30% level of incorporation as compared to control ($p<0.05$).

Scores for the sensory colour and appearance, texture, and flavour of the control and experimental biscuits slightly decreased during storage (Table 3). However, no undesirable changes were noticed in the biscuits. Significant changes were observed in the overall acceptability of biscuits after 30 days of storage. Moisture content of the control and experimental biscuits slightly increased with the level of incorporation of WPC over the storage period (Table 3). However, the moisture and acidity of the extracted fat of all the biscuits were within the range of specifications (6 and 1.5%) for protein enriched biscuits (ISI 1986). The increase in the acidity of extracted fat of the biscuits could be due to increased moisture content, which promoted fat oxidation during storage, thereby increasing the fat acidity. Similar results of increase in moisture were reported by Bassi and De (1973) when HPB made from sodium caseinate were packed in polythene pouch and stored at room temperature (30-35$^0$C) for 50 days. Singh et al (2000) also reported similar observations in the storage of soy-fortified biscuits. The total mesophilic bacterial and yeast and mold counts increased with the storage period (Table 3). However, the total bacterial counts of all the samples were within specified limits of 50,000 cfu/g for protein-enriched biscuits (ISI 1986).

## Conclusion

Protein-enriched biscuits can be prepared by incorporating WPC up to 30% in the formulation without affecting their overall quality. There was a 3-fold increase in protein content of biscuits with 30% incorporation of WPC. The cutting and compression strengths of the biscuits increased with the increase in the level of WPC. These protein-enriched biscuits can be stored at ambient temperatures (30-35 $^0$C) for 60 days without any undesirable changes in the sensory attributes. Hence, development of such protein-enriched biscuits will not only improve the nutritional status of the general population but also help vulnerable groups suffering from protein-energy malnutrition.

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