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Nutritional assessment of cookies supplemented with defatted wheat germ

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Abstract

Replacement of wheat flour with defatted wheat germ (DFWG) at levels of 0–25% was investigated for its effect on functional and nutritional properties of cookies. The crude protein content of DFWG was as high as 27.8% with a highly valuable amino acid profile, rich in essential amino acids, especially lysine (2.32 g/100 g). The physicochemical and sensory evaluation of cookies, revealed that up to 15% substitution of wheat flour with DFWG produced acceptable cookies similar to the control (100% wheat flour) cookies. The protein quality of the cookies was assessed through weanling albino rats by feeding a diet of cookies for 10 days, which was formulated to supply 10% protein, with a casein diet as a control. The cookies containing 15% DFWG, were best regarding protein bioavailability in rats. The protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and true digestibility (TD) differed significantly among diets containing cookies with 0–10% DFWG, and casein diet when fed to rats. Diets containing 15% DFWG have values, of these parameters, similar to the casein diet.

Keywords: Defatted wheat germ; Cookies; Physicochemical composition; Protein quality

1. Introduction

The protein foods available commercially are obtained from a range of animal and plant sources and are used as functional ingredients (Periago & Vidal, 1998). The increased costs and limited supplies of animal proteins, have necessitated contemporary research efforts geared towards the study of food properties and potential utilization of protein from locally available food crops, especially from under-utilized or relatively neglected high protein oilseeds and legumes (Enujiugha & Ayodele-Oni, 2003). Wheat germ protein is reported to have a high nutritive value, comparable to that of animal proteins. In the wheat grain, most nutrients, with the exception of starch, are concentrated in germ but most of this is generally used in animal feed formulations, due to which the precious wheat germ source has not been amply, rationally, and efficiently utilized (Shurpalekar & Rao, 1977). The germ provides three times as much protein, seven times as much fat, fifteen times as much sugars and six times as much mineral content than does wheat flour (Rao, Kumar, Rao, & Shurpaleker, 1980).

Defatted wheat germ, after extraction of valuable wheat germ oil, is a high nutritive value protein material, which contains about 30% protein (Ge, Sun, Ni, & Cai, 2000). Wheat germ protein has been classed with effectively superior animal proteins and is rich in amino acids, especially the essential amino acids, lysine, methionine, and threonine, in which many cereals are deficient (Yiqiang, Aidong, & Tongyi, 1999). Therefore, wheat germ is a potential nutritious food supplement; in particular, defatted wheat germ is a source of natural high grade protein, which makes it a good enrichment component for many foods (Moss, Murray, & Stenvert, 1984).

The enrichment of cereal-based foods with oilseed and legume protein has received considerable attention. Wheat bread and cookies are widely accepted and consumed in

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many developing countries and therefore offer a valuable supplementation vehicle for nutritional improvement; however, cookies have been suggested as a better use of composite flour than bread because of their ready-to-eat form, wide consumption and relatively long shelf-life (Lorens, Dilsaver, & Wolt, 1979). Protein enriched cookies are attractive for target areas, such as child-feeding programmes, low-income groups and disaster relief operations (Claughton & Pearce, 1989). Cookies with these characteristics have been produced from blends of wheat and cowpea (McWatters, Ouedraogo, Resurrection, Hung, & Philips, 2003) or soybean and wheat (Shrestha & Noomhorm, 2002). However, there is no information on the use of DFWG in cookie-making. In this study, we attempted to assess the suitability of DFWG utilization for improvement in quality and nutritive value of cookies, and to strengthen the utilization of wheat germ.

2. Materials and methods

2.1. Raw materials and treatment

Wheat was purchased from the Ayub Agricultural Research Institute (AARI), Faisalabad. Straight grade flour was prepared by a UDY cyclone mill in the Institute of Food Science and Technology, University of Agriculture, Faisalabad. After milling of wheat, flour samples were packed in polypropylene bags and stored at room temperature for further study. Raw wheat germ was procured from Sunny Flour Mills, Lahore, Pakistan. Wheat germ contains several enzymes, such as dipeptidase, proteinase, lipase, lipoxidase and phytase. In order to stabilize it, wheat germ was treated by 'Characteristic Far Red' ray technology to kill its enzymes, during which the temperature was 130–160 °C for 20–25 min. Treatment of wheat germ, under these conditions, is reported to be sufficient enough to deactivate enzyme activity (Zwingelberg & Fretzdorff, 1996).

2.2. Preparation of DFWG flour

Wheat germ oil was extracted by a solvent extraction technique, using *n*-hexane (BP. 68 °C). DFWG remaining after oil extraction was crushed and passed through a 200-mesh sieve to obtain DFWG flour, which was used as our experimental material.

2.3. Blends formulation and preparation of cookies

Blends of wheat flour and DFWG flours containing 0%, 5%, 10%, 15%, 20% and 25% DFWG flour, on a replacement basis, were prepared. The choice of these levels was based on the report of Dreuiter (1978) that the maximum level of wheat flour substitution that would produce an acceptable baked product was 25%. They were then packed in polyethylene bags, sealed and stored in a freezer (0–5 °C) until required.

Cookies were prepared according to the procedure described by McWatters et al. (2003) with slight modifications. The basic ingredients used were 380 g of flour blend, 100 g vegetable shortening, 225 g of granulated cane sugar, 21 g of beaten whole egg, 3.75 g of salt, and 1.8 g of baking powder. The dry ingredients were weighed and mixed thoroughly in a bowl by hand for 3–5 min. Shortening was added and rubbed in until uniform. The egg was added and dough was thoroughly kneaded in a mixer for 5 min. The dough was rolled thinly on a sheeting board to a uniform thickness (8.0 mm) and cut out using a round scorn cutter to a diameter of 35.0 mm. The cut out dough pieces were baked on greased pans at 160 °C for 15 min in a baking oven. The prepared cookies were cooled to room temperature (30 ± 2 °C) and packed in high density polyethylene bags.

2.4. Chemical composition of flours and cookies

Amino acid content of DFWG was determined by using an autoanalytic apparatus according to the method of Yu (1994). All reagents used were of analytical grade (BDH Chemicals, Poole, UK).

The moisture, crude protein, crude fat, total ash and crude fibre contents of flours and cookies were determined by AACC (2000). Nitrogen-free extract (NFE) was calculated by difference. The factors, $n = 5.70$ (for wheat flour), and $n = 6.25$ (for cookies) were used for conversion of nitrogen to crude protein. Minerals, including, calcium, iron and potassium, were determined using an atomic absorption spectrophotometer, AAS (Model 372, Perkin–Elmer Ltd., Beaconsfield, UK) by dry-ashing, according to the procedure of the AACC (2000). All reagents used were of analytical grade (BDH Chemicals, Poole, UK).

2.5. Physical and organoleptic evaluation of cookies

Physical parameters, including, diameter, height, weight and hardness, of cookies were measured on three replicates and mean values recorded. Cookie diameters and heights were measured with a vernier caliper. Weights were determined using a Mettler digital top loading balance (PC 400; Mettler, Buchi Switzerland).

Cookies were evaluated for colour, flavour, texture and overall acceptability, according to the preference method of Ihekoronye and Ngoddy (1985). Ten (trained) judges participated in the sensory evaluation of the cookies on a 5-point hedonic scale. The coded cookie samples were randomized and presented to the judges in the midmorning on white plates in the sensory evaluation laboratory, Institute of Food Science and Technology, University of Agriculture, Faisalabad.

2.6. Biological evaluation of protein quality

Based on the results of physicochemical and sensory evaluation of cookies, four best treatments, including control (0% DFWG), were selected for protein quality evaluation by a rat bioassay. Protein diets were formulated using flours from cookie samples containing 0% (diet A, control), 5% (diet B), 10% (diet C), and 15% (diet D). Diets were prepared according to AOAC (1995) formulation. Their compositions are shown in Table 1. Weanling male albino rats, 28 days old and weighing between 42 and 45 g, were grouped by randomized block design into six groups on the basis of weight, such that mean initial weights did not differ by more than ±0.5 g. Each group consisted of 10 rats and they were housed in individual wire-bottom cages that allowed for easy faecal collection and the measurement of food intake. The temperature of the animal room was 27 ± 1 °C with alternate 12 h. periods of light and dark. One group of 10 rats was fed casein (diet E). The other was fed a protein-free diet (F), which consisted entirely of the basal diet, while the remaining four groups were fed the experimental diets (A–D). The diets were supplemented with vitamins and minerals to target requirements (Grant, Dorward, & Pusztai, 1993). Rats were given free access to diet and water. Daily records, of weight gain or loss, food and protein intakes and urinary and faecal output by the rats, were taken. Urinary and fecal nitrogen samples from the appropriate test diet group were collected and analyzed by the Kjeldahl method (AOAC, 1995).

The data obtained from this experiment were used to calculate protein efficiency ratio (PER), true digestibility (TD), biological value (BV) and net protein utilization (NPU) by following the procedures outlined by Pellet and Young (1980). True digestibility is the apparent digestibility corrected for metabolic N in the fecal material, and biological value is the percentage of absorbed N that is retained by the rats (after correction for urinary N and endogenous urinary N). Net protein utilization is derived from true protein digestibility × biological value per 100.

2.7. Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) according to Steel, Torrie, and Dickey (1997). Means were separated by least significant difference (LSD). Significance was accepted at $p \leqslant 0.05$.

Table 1
Composition of experimental diets fed to rats

Diets[a]	Ingredients of diets (g/100 g)					
	Cookies	Corn oil	Mineral mixture	Vitamin mixture	Corn starch	Casein
A	83.7	10	2.5	1	2.8	–
B	78.2	10	2.5	1	8.3	–
C	73.7	10	2.5	1	12.8	–
D	69.1	10	2.5	1	17.4	–
E	–	10	2.5	1	76.5	10
F	–	10	2.5	1	86.5	–

[a] Prepared from cookies containing 0% (A), 5% (B), 10% (C), 15% (D) defatted wheat germ flour; casein (E) and protein-free diet (F).

3. Results and discussion

3.1. Amino acid profile of DFWG

The amino acid profile of DFWG is shown in Table 2. The amino acid varieties of DWGP were inclusive, and especially rich in essential amino acids and limited amino acids for the human body. Furthermore, the composition ratio of essential amino acids of DFWG, comparable to egg and milk proteins, was basically close to the model value issued by the FAO/WHO with good amino acid equilibrium, which was very significant from a nutritional point of view.

3.2. Chemical analyses of flours and cookies

The chemical compositions of wheat flour and DFWG flour used for cookies preparation are shown in Table 3. Crude protein (28.9%), crude fibre (5.35%), ash (4.52%) and mineral content are higher in the case of DFWG flour than of wheat flour. A highly significant ($P \leqslant 0.05$) difference was also obtained between control and DFWG-enriched cookies for these chemical parameters (Table 4). Results of the chemical analysis of cookies revealed that protein contents of the cookies, prepared from defatted wheat germ flour blends, were significantly higher than the protein content of control cookies. The protein content of the cookies prepared from these blends was also higher (12.7–16.2%) than those (6–12%) reported for conventional cookies (Shrestha & Noomhorm, 2002).

Table 2
Amino acid content of DFWG (g/100 g)

Amino acid	Content	Amino acid	Content
Tryptophan	0.201	Valine	1.40
Threonine	0.928	Arginine	4.76
Isoleucine	1.73	Histidine	0.838
Leucine	1.11	Alanine	1.66
Lysine	2.32	Aspartic acid	1.63
Methionine	0.248	Glutamic acid	5.09
Phenylalanine	1.04	Glycine	1.47
Tyrosine	0.779	Serine	1.43
Total			26.6

Table 3
Chemical compositions of flours used for cookies preparation

Components	Wheat flour	DFWG flour
Moisture (%)	10.0 ± 0.4	13.2 ± 0.3
Crude protein (%)	11.5 ± 0.5	27.8 ± 0.4
Crude fat (%)	0.92 ± 0.1	0.05 ± 0.01
Crude fibre (%)	0.90 ± 0.15	5.35 ± 0.3
Ash (%)	0.74 ± 0.1	4.52 ± 0.2
NFE (%)	76.7 ± 1.2	58.0 ± 1.0
Calcium (mg/100 g)	32.9 ± 0.7	45.9 ± 0.3
Iron (mg/100 g)	0.3 ± 0.1	7.36 ± 0.8
Potassium (mg/100 g)	125 ± 1.1	1050 ± 2.0

Table 4
Chemical compositions and mineral content of DFWG-supplemented cookies

Components	Defatted wheat germ flour level in cookies (%)					
	0	5	10	15	20	25
Moisture (%)	8.37 ± 0.10^{b}	8.50 ± 0.09^{ab}	8.66 ± 0.08^{ab}	8.79 ± 0.08^{ab}	8.91 ± 0.08^{ab}	9.02 ± 0.11^{a}
Crude protein (%)	11.8 ± 0.13^{f}	12.7 ± 0.14^{e}	13.6 ± 0.15^{d}	14.5 ± 0.16^{c}	15.4 ± 0.16^{b}	16.2 ± 0.17^{a}
Crude fat (%)	14.15 ± 0.15^{a}	14.09 ± 0.12^{ab}	14.07 ± 0.12^{ab}	14.02 ± 0.11^{ab}	13.98 ± 0.11^{ab}	13.94 ± 0.10^{ab}
Crude fibre (%)	2.33 ± 0.06^{d}	2.56 ± 0.07^{cd}	2.76 ± 0.07^{bcd}	3.01 ± 0.07^{abc}	3.19 ± 0.08^{ab}	3.27 ± 0.08^{a}
Ash (%)	0.76 ± 0.02^{e}	0.95 ± 0.03^{de}	1.13 ± 0.04^{cd}	1.29 ± 0.05^{bc}	1.50 ± 0.05^{ab}	1.690 ± 0.05^{a}
NFE (%)	62.6 ± 1.90^{a}	61.2 ± 1.60^{b}	59.8 ± 1.50^{c}	58.4 ± 1.30^{d}	57.0 ± 1.22^{e}	55.8 ± 1.21^{f}
Calcium (mg/100 g)	50.1 ± 1.20^{f}	50.8 ± 1.30^{e}	51.3 ± 1.30^{d}	52.0 ± 1.40^{c}	52.4 ± 1.45^{b}	53.1 ± 1.46^{a}
Iron (mg/100 g)	1.85 ± 0.05^{e}	2.21 ± 0.06^{de}	2.48 ± 0.06^{cd}	2.71 ± 0.06^{bc}	3.01 ± 0.07^{ab}	3.22 ± 0.07^{a}
Potassium (mg/100 g)	105 ± 3.50^{f}	152 ± 4.05^{e}	196 ± 4.50^{d}	238 ± 5.70^{c}	276 ± 6.05^{b}	306 ± 6.9^{a}

Means in the same row bearing the same letter are not significantly different ($p \leqslant 0.05$).

All the cookies supplemented with DFWG were found to be nutritious on the basis of these parameters. This was because the consumption of about 100 g of each product formulation would provide more than half of the recommended daily requirement for protein (25–30 g/day), as recommended by FAO/WHO (1973) for children aged between 5 and 19 years. This fact suggests that cookies supplemented with DFWG may be useful as food supplements for the alleviation or prevention of protein malnutrition in developing countries.

Addition of DFWG resulted in an increase in ash values of cookies up to 1.69% and crude fibre content up to 3.27%, while crude fat content was decreased to 13.94% (25% of DFWG level). There were no significant differences in moisture contents of cookies. Supplementation of cookies with DFWG also significantly ($P \leqslant 0.05$) increased the levels of calcium, iron and potassium to 53.1, 3.22 and 306 mg/100 g, respectively (Table 4).

3.3. Physical characteristics of cookies

Data on the physical characteristics of cookies are presented in Table 5. There were no significant ($P \leqslant 0.05$) differences between the values obtained for spread ratio and hardness of cookies supplemented with 5–15% DFWG flour and the control (100% wheat flour) cookies: however, these parameters decreased significantly in the case of cookies supplemented with more than 15% DFWG. Blends containing more than 15% DFWG flour produced softer cookies that required less force to compress. In general, cookies made with wheat–DFWG flour blends had reduced heights and diameters and increased weights; these effects increased with increasing level of replacement of wheat flour with DFWG flour. These results were similar to those reported for cookies prepared from wheat–cowpea (McWatters et al., 2003) and wheat–soybean (Shrestha & Noomhorm, 2002) flour blends. There are several views on the mechanisms by which the diameter of cookies (i.e. spread) is reduced when wheat flour is supplemented with non-wheat flours. McWatters (1978) reported that rapid partitioning of free water to hydrophilic sites during mixing increased dough viscosity, thereby limiting cookie spread. However, it has been suggested that spread ratio is affected by the competition of ingredients for the available water; flour or any other ingredient, which absorbs water during dough mixing, will decrease it (Fuhr, 1962).

3.4. Organoleptic evaluation of cookies

The effects of DFWG flour on the sensory quality of cookies are summarized in Table 6. Acceptable cookies that closely resembled the control (100% wheat flour) cookies were produced from wheat flour containing up to 15% DFWG flour. The results of sensory analysis showed that scores assigned by the judges for texture were in good agreement with the measurements derived from the physical (hardness) test. The low overall acceptability of the cookies from blends containing more than 15% DFWG flour was attributed, by the panellists, to a crumbly texture, a beany flavour and darkening. Colour darkening of cookies is attributed to sugar caramelization and the Maillard reactions between sugars and amino acids (Alobo, 2001).

Table 5
Physical characteristics of DFWG-supplemented cookies

Parameters	Defatted wheat germ flour level in cookies (%)					
	0	5	10	15	20	25
Weight (g)	10.76 ± 0.10^{c}	10.79 ± 0.09^{c}	11.09 ± 0.12^{b}	11.12 ± 0.11^{b}	12.46 ± 0.11^{a}	12.49 ± 0.13^{a}
Diameter (cm)	9.35 ± 0.08^{a}	9.05 ± 0.10^{b}	7.35 ± 0.08^{c}	6.98 ± 0.06^{d}	4.30 ± 0.04^{e}	4.10 ± 0.04^{f}
Height (cm)	1.30 ± 0.02^{a}	1.27 ± 0.03^{a}	1.25 ± 0.02^{a}	1.00 ± 0.001^{b}	0.75 ± 0.02^{c}	0.72 ± 0.01^{c}
Spread ratio	7.19 ± 0.07^{a}	7.13 ± 0.06^{a}	7.10 ± 0.05^{a}	7.08 ± 0.05^{a}	5.73 ± 0.05^{b}	5.69 ± 0.04^{b}
Hardness (N)	56.51 ± 1.20^{a}	55.96 ± 1.10^{a}	54.68 ± 1.20^{a}	53.91 ± 1.05^{a}	47.89 ± 0.90^{b}	43.56 ± 0.7^{c}

Means in the same row bearing the same letter are not significantly different ($p \leqslant 0.05$).

Table 6
Organoleptic characteristics of DFWG-supplemented cookies

Parameters	Defatted wheat germ flour level in cookies (%)					
	0	5	10	15	20	25
Colour	6.80 ± 0.11^{a}	6.30 ± 0.09^{b}	6.40 ± 0.08^{ab}	6.15 ± 0.08^{b}	5.05 ± 0.07^{c}	4.65 ± 0.07^{c}
Flavour	7.50 ± 0.12^{a}	7.50 ± 0.10^{a}	7.45 ± 0.10^{ab}	7.30 ± 0.10^{b}	5.05 ± 0.05^{c}	4.40 ± 0.05^{d}
Texture	6.80 ± 0.10^{a}	6.00 ± 0.08^{b}	5.80 ± 0.07^{c}	5.75 ± 0.07^{c}	3.55 ± 0.05^{d}	3.40 ± 0.05^{d}
OA[A]	7.65 ± 0.10^{a}	7.35 ± 0.11^{a}	7.50 ± 0.10^{a}	7.65 ± 0.10^{a}	4.85 ± 0.04^{b}	3.90 ± 0.04^{c}

Means in the same row bearing the same letter are not significantly different ($p \leqslant 0.05$).
[A] Overall acceptability.

Table 7
Protein quality parameters of diets containing experimental cookies fed to rats

Diets[A]	Protein quality parameters[B]					
	Weight gain (g/10days)	Daily feed intake (g)	PER	NPU (%)	BV (%)	TD (%)
A	$12.50 \pm 0.8e$	12.30 ± 0.5^{a}	1.11 ± 0.03^{c}	47.4 ± 2.10^{d}	63.2 ± 2.80^{d}	69.5 ± 2.35^{d}
B	27.23 ± 1.0^{d}	12.40 ± 0.5^{a}	1.63 ± 0.05^{b}	50.2 ± 2.40^{c}	65.8 ± 2.80^{c}	75.8 ± 3.10^{c}
C	29.80 ± 1.2^{c}	12.62 ± 0.6^{a}	1.72 ± 0.05^{b}	61.1 ± 2.30^{b}	78.6 ± 2.82^{b}	88.4 ± 3.25^{b}
D	37.42 ± 1.3^{b}	12.31 ± 0.7^{a}	2.78 ± 0.07^{a}	63.3 ± 2.30^{a}	80.6 ± 3.05^{b}	90.6 ± 3.30^{a}
E	40.66 ± 1.3^{a}	12.34 ± 0.7^{a}	2.88 ± 0.08^{a}	65.1 ± 2.81^{a}	85.4 ± 3.25^{a}	93.5 ± 3.30^{a}

PER, protein efficiency ratio; NPU, net protein utilization; BV, biological value; TD, true digestibility.
Means in the same row bearing the same letter are not significantly different ($p \leqslant 0.05$).
[A] Prepared from cookies containing 0% (A), 5% (B), 10% (C) and 15% (D) defatted wheat germ flours; E, casein (control).
[B] Values are averages (10 rats per diet) ± standard deviation.

Flavour was found to be a prominent factor in determining acceptability of DFWG-supplemented cookies. Panellists described the cookies containing more than 15% DFWG flour as having an aftertaste and a beany flavour. This implies that there is a need to seek preliminary processing methods (e.g. heat treatment) to improve the performance of these flours. Heat treatment (steaming for 30 min at 100 °C) was found to be effective for improving the flavour of cowpea flour used in cookie-preparation (McWatters, 1985).

3.5. Biological evaluation of protein quality

The cookies containing 5%, 10% and 15% DFWG along with control (0% DFWG) were found to be the best, based on the results of physicochemical and sensory evaluation, and were subjected to protein quality evaluation through a rat bioassay. The results of rats feeding trials, conducted for the determination of protein quality of cookies, compared with casein, are presented in Table 7. These results provide the first report of an improvement in the nutritive value of cookies as a result of DFWG addition. Rats fed on control diet had the lowest body weight gain (12.34 g), significantly different ($p \leqslant 0.05$) from the DFWG-supplemented diets (B, C and D) and the casein diet (E). Daily feed intakes were not significantly different ($p \leqslant 0.05$) among rat groups (Table 7).

The relatively higher essential amino acid balance in DFWG-supplemented cookies seems to favour these diets to give better weight gain than the control diet. Diets formulated from cookies supplemented with DFWG at the 10–15% levels (C and D) gave significantly ($P \leqslant 0.05$) higher PER, NPU, BV and TD than did the A (control) and B (5% DFWG) diets.

In short, increases were found in the two directly measured parameters, BV and TD, and in the derived value for NPU with the increase in DFWG level in cookies. Rats fed control diet (100% wheat flour cookies) showed poor protein quality indices, such as low NPU (47.4%), BV (63.2%) and TD (69.5%). Diets D and E had significantly ($P \leqslant 0.05$) higher values of PER (2.78 and 2.88, respectively) than had the remaining diets. The results suggested that the nutritional quality of cookies improved with a 10–15% supplementation of DFWG flour. However, cookies supplemented with DFWG at the 15% level were nutritionally comparable with diets based on casein. It may be concluded that the differences in protein quality parameters are because of the essential amino acid content of DFWG protein which is higher than the control diet containing 100% wheat flour.

The results of this study have shown that highly nutritious cookies can be prepared by supplementing wheat flour with DFWG flour at 5–15% levels, with increased crude protein, calcium, potassium and iron contents. Also, diets based on supplemented cookies containing 15% DFWG flour were nutritionally comparable with a diet based on casein, indicating that the under-utilized high protein wheat germ could be processed into value-added products and used to combat malnutrition.

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