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The in vitro protein digestibility, microbiological quality and gelatinization behaviour of macaroni as affected by cowpea flour addition

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Abstract

Cowpeas were germinated, fermented, cooked, ground to flour and added to standard durum wheat semolina at 20% (w/w) level for macaroni production. Macaroni samples were analysed for in vitro protein digestibility, microbial count (total bacteria, mould and yeast) and gelatinization behaviour over a 6-month storage period at room temperature (<25 °C). Starch gelatinization behaviour of the samples was analysed using differential scanning calorimetry. Supplementing semolina with cowpea flour did not have a significant affect on in vitro protein digestibilities or aerobic plate counts of macaroni samples ($p < 0.05$). There was a small but significant increase in mold and yeast counts after 6 months of storage in cowpea treated samples. Two endothermic peaks were observed with significant differences in ΔH values of control and cowpea treated macaroni samples. The transition peak (T_p) temperatures were in the range of 66.9–67.9 and 86.9–100.4 °C for the first and second peaks, respectively. The transition enthalpies (ΔH) were in the range 2.41–4.21 and 1.71–3.86 J/g for the first and second peaks, respectively.

Keywords: Cooking; Differential scanning calorimetry; Digestibility; Fermentation; Germination; Starch gelatinization

1. Introduction

Dry cowpea seeds (*Vigna ungiuculata*) contain approximately 10–12% moisture, 24–26% protein, 1.3–1.5% fat, 56–58% carbohydrate, 4.4–5.0% fibre and 3.6–4.0% ash (Deshpande & Damodaran, 1990). They are an inexpensive source of protein with some indigestible components, as well as some antinutritional factors, such as trypsin inhibitors (McWatters, Enwere, & Fletcher, 1992). Various processing methods, such as soaking, dehulling (Phillips, Chinnan, Branch, Miller, & McWatters, 1988) germination, cooking (Giami, Akusu, & Emelike, 2001) and fermentation (Zamora & Fields, 1979) have been reported to achieve improvements in nutritional value and digestibility (Kiers, Nout, & Rombouts, 2000).

The biological utilization of protein, in any food product, is primarily dependent on digestibility. According to Rathi, Kawatra, and Sehgal (2004), the in vitro protein digestibility of pasta prepared from native pearl millet was relatively low and it was attributed to the presence of considerable amounts of some antinutrients in unprocessed or native pearl millet. It was reported that in vitro digestibilities of legumes were increased during cooking and fermentation (Kiers et al., 2000).

There have been many studies on the improvement of nutritional quality of macaroni by adding protein-rich sources (Corta, Coelho, & Bicudo, 1990; Schmidt, Rodrick, & Turner, 2003). Overall quality of durum wheat macaroni is influenced primarily by the properties of

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the protein and starch fractions. The functional properties of cowpea make it suitable for incorporation into protein-rich food products (Abbey & Ibeh, 1988). Differential scanning calorimetry (DSC) is a thermo-analytical technique for monitoring changes in physical and chemical properties of materials as a function of temperature. More studies have been reported on thermal behaviour of starch and protein, as isolated preparations, than on their thermal behaviour as they occur in a food system (Henshaw, McWatters, Akingbala, & Chinnan, 2003).

The objective of this study was to incorporate cowpea flour into standard macaroni formulation to produce a product with improved nutritional value. It was also aimed to monitor the changes of in vitro protein digestibility (IVPD), microbiological properties, and thermal properties of cowpea treated samples over a 6-month storage at room temperature.

2. Materials and methods

2.1. Cowpeas

Dry cowpeas were purchased from a local market in Gaziantep, Turkey. The seeds were hand-sorted to remove wrinkled, moldy seeds and foreign material.

Control (unprocessed) cowpea flour. Cowpeas were soaked in distilled water for 10 h at room temperature, decorticated manually, dried at 60 °C for 8 h and milled to flour by a lab scale hammer mill to pass a 20 mesh screen.

2.2. Germinated cowpea flour

Cowpeas were soaked in distilled water for 10 h at room temperature. The hydrated seeds were spread between clean moist cotton sheets over a metal tray. Germination took place at 30 °C for 24 h in a thermostatically controlled oven. Germinated cowpea roots and testa were rubbed off by hand and seeds were dried at 60 °C for 8 h in an oven, followed by milling to flour as above.

2.3. Fermented cowpea flour

Control (unprocessed) cowpea flour was mixed with water (1:4, w/w) to form a slurry which was left to ferment at 30 °C for 24 h by its indigeneous flora. The surface of the slurry was covered with aluminium foil to prevent dehydration. Fermented cowpea slurries were dried at 60 °C for 8 h and milled to flour as described above.

2.4. Cooked cowpea flour

Soaked and decorticated cowpeas, as above, were cooked in boiling excess water for 10 min and dried at 60 °C for 12 h, followed by milling as before.

2.5. Macaroni production

Macaroni samples were prepared using a pilot scale macaroni-making device consisting of a mixer-press and a drier (Namad-Microimpianti Lab.Cereal C. Garampi, Italy). Processed cowpea flours (namely, control, germinated, fermented, cooked) were added to durum wheat semolina at 20% (w/w) level, based on the semolina used. The moisture content of the blends before drying was kept at 33% wb by addition of water. The final moisture content of the product, after drying, was at most 10–12%. The drying took place at 40 °C and 60% relative humidity in the dryer. The thicknesses of the macaroni samples were approximately 1.7 mm after the dryer. The samples were kept in polyethylene bags until used.

2.6. Sample composition

Cowpea flours and macaroni samples were analysed for their crude protein and moisture contents using standard methods (Anonymous, 1990). The Kjeldahl method was used to determine crude protein content of the samples with conversion factors of 6.25 and 5.70 for cowpea flour and semolina, respectively. All tests were done in triplicate and results were expressed on a dry basis. Chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO).

2.7. Microbial analysis of macaroni samples

A 25.0 g macaroni sample was weighed aseptically into a sterile glass beaker (500 ml) and mixed with 225 ml of a sterile peptone–water solution (0.1%), using a magnetic stirrer for 20 min, to obtain a homogenized sample (Jay, 1986).

2.8. Aerobic plate count (APC)

The aerobic plate count was done using the aerobic spread plate count method described by Jay (1986). Aerobic plate count agar (PCA; Merck, Darmstadt, Germany) was used for the analysis.

Dilutions of the sample were prepared from 1 to 1×10^{-7} and an amount of 0.2 ml from every dilution was transferred onto a corresponding labelled petri dish and spread- plated over the agar surface. Inoculated PCA plates were incubated at 35 °C for 24–72 h. The plates with less than 300 colony forming units (CFU) were counted and the average value was taken after a duplicate count. The number of CFUs was multiplied by the dilution factor and divided by the inoculation amount in order to determine the CFUs per gramme of macaroni. The CFU numbers were transformed into corresponding logarithmic numbers.

2.9. Mold and yeast counts

Mold and yeast counts were done by the aerobic spread plate count method described by Jay (1986). Potato dextrose agar (PDA; Merck, Darmstadt, Germany) was used for the analysis. 0.2 ml of the dilution was transferred onto a corresponding labelled plate and spread-plated over the agar surface. Inoculated PDA plates were incubated at 25 °C for 2–5 days. The log CFU were calculated as described above.

2.10. In vitro protein digestibility

Uncooked macaroni samples were investigated for their in vitro protein digestibilities by the modified methods of Hsu, Vavak, Satterlee, and Miller (1977) and Dahlin and Lorenz (1993). Fifty millilitres of aqueous protein suspension, based on crude protein content (6.25 mg protein/ml), were allowed to rehydrate for 60 min at 5 °C with intermittent mixing. After rehydration, samples were placed in a 37 °C water bath and the pH was adjusted to 8.00 using 0.1 N NaOH and/or 0.1 N HCl, while stirring. Lyophilized, crystallized trypsin (Sigma Chemical Co., St Louis, MO), at a concentration of 1.6 mg/ml, was maintained in an ice bath and the pH was adjusted to 8.00 with 0.1 N NaOH and/or 0.1 N HCl. Five milliliters of enzyme solution were then added to the protein suspension, which was being stirred at 37 °C. The trypsin had an activity of 13766 BAEE units/mg protein. A rapid decline in pH occurred immediately. The pH drop was recorded 15 s after enzyme addition and at 1 min intervals for 10 min. Triplicate analyses were performed for each sample. The enzyme solution was freshly prepared before each series of the test. The percent protein digestibility (Y) was calculated in the following equation (Hsu et al., 1977):

$$Y = 210.4 - 18.1x, \quad (1)$$

where x is the change in pH after 10 min.

2.11. Differential scanning calorimetry

A Perkin–Elmer DSC 6 (Perkin–Elmer Ltd., Norwalk, CT) instrument was used to analyse dry macaroni samples. The instrument was calibrated with indium, and the instrument sensitivity was 0.5 mcal/s. Ground macaroni samples were sieved through a 425 μm mesh screen. Samples of known moisture content were carefully wetted with distilled water to give a paste with a ratio of dry sample to water of 1:3 (Güler, Köksel, & Ng, 2002). Samples were weighed into standard aluminium differential scanning calorimeter (DSC) pans, sealed and allowed to equilibrate for 1.5 h prior to analysis (Pınarlı, İbanoğlu, & Öner, 2004). A sample size of 12 ± 3 mg was used. The samples were heated at a rate of 5 °C/min from 30 to 130 °C with nitrogen flushing rate of 40 cm^3/min. A sealed empty pan was used as the reference. The starch gelatinization characteristics in the DSC thermogram can be explained by various defined temperatures. By using the instrument's analysis software programme, for each endotherm, the onset temperature (T_o), the peak temperature (T_p) and enthalpy (ΔH, the amount of energy required for gelatinization) were computed (Krueger, Knutson, Inglett, & Walker, 1987). Enthalpies were reported on a dry sample basis.

2.12. Statistical analyses

All determinations were done in triplicate, and means ± standard deviation (SD) values were calculated. A commercial software programme (SPSS 11.0 SPSS) was used to perform statistical analyses. Data were assessed by analysis of variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at probability $p \leqslant 0.05$ throughout the analysis.

3. Results and discussion

3.1. Background

In our preliminary work (Herken, 2005), macaroni was produced from 10%, 15% and 20% cowpea flour. According to sensory evaluations of macaroni samples, most of the total sensory scores of 20% cowpea flour supplemented macaroni samples were not significantly different from the control (produced from 100% wheat semolina) ($p < 0.05$). The ash content of sample with 20% cowpea flour has been found to be within acceptable limits (Anonymous, 1989). Therefore, our present study was carried out on 20% cowpea flour treated macaroni samples only.

3.2. Microbial count

Macaroni production involves a controlled drying step at warm temperatures which, if not properly controlled, allows the extensive growth of microorganisms (Swartzentruber, Rayne, Wentz, Barnard, & Read, 1982). Most of the microbiological studies of macaroni products have focussed on pathogen growth during production rather than on the general microbiological quality. Macaroni is generally regarded as a microbiologically safe product, as it has relatively low water activity (i.e., <0.50). Swartzentruber et al. (1982) found that the geometric means of aerobic plate counts for macaroni and noodle products were 520 and 1400 per g, respectively; means of yeast and mold counts were 72 per g for macaroni and 100 per g for noodles. These values were within safe limits (Rayman, Weiss, Riedel,

Charbonneau, & Jarvis, 1981). During cowpea flour production, some processes, especially fermentation, may lead to contamination by pathogens which can then survive for a period. Therefore, aerobic plate counts (APC) and mold and yeast counts were conducted for the control and enriched macaroni samples over a 6-month period and compared with the standards.

The results showed that aerobic plate counts (APC) of the samples were not significantly different from each other ($p < 0.05$) (Table 1) with no significant increase during the 6-month period. For cereals and legumes, soaking and cooking process were both found to leach out or decrease some undesirable factors and reduce the in situ microbial contaminants, especially non-sporulating bacteria and molds. In general, the fermentation increased the shelf life of cereals and legumes (Wang & Hesseltine, 1981). Our results show that addition of cowpea flour did not affect microbiological safety of samples.

The mold and yeast count of the control and cowpea flour supplemented macaroni samples increased significantly during storage ($p < 0.05$) (Table 2). The APC and mold and yeast counts of the macaroni samples were both below the safety limit of the macaroni standards, which are 5 and 2.699 log CFU/g, respectively (Anonymous, 1989).

3.3. In vitro protein digestibility

The in vitro protein digestibility (IVPD) values of macaroni samples studied are given in Table 3. Results show that adding cowpea flour to macaroni at 20% did not change the IVPD values of the sample ($p < 0.05$). It is also seen from Table 3 that a 6-month storage at room temperature did not affect the IVPD of the samples ($p < 0.05$). It was observed that, during IVPD analysis, the pH dropped suddenly for the first 2 min and then decreased more slowly after 4 min of incubation, possibly because the substrate to enzyme ratio declines. During cereal-enzyme incubation, hydrolysis occurs and amino acids are released from the peptide chain, resulting in decrease of pH, a marker for increased protein digestibility (Dahlin & Lorenz, 1993). The IVPD results were between 78.3% and 80.2%, which were not significantly different from each other ($p < 0.05$).

The results of studies on the effect of germination on in vitro protein digestibility. According to a study by Nnanna and Phillips (1989) IVPD of cowpea was not improved significantly by germination nor by decortication but was improved by cooking. Njintang, Mbofung, and Waldron (2001) revealed that protein digestibility of bean flours increased with germination and drying temperature. Fermentation and germination significantly ($p < 0.05$) increased protein digestibility of fluted pumpkin seed flours (Giami & Isichei, 1999). Casagrandi, Canniatti-Brazaca, Salgado, Pizzinato, and Novaes (1999) found that the addition of pigeon pea flour to macaroni decreased protein digestibility by approximately 4%. However, Rayas-Duarte, Mock, and Satterlee (1996) found that spaghetti, made from lupin flours, retained overall digestibility compared to the control.

Table 1
Aerobic plate counts of the macaroni samples enriched with 20% cowpea flour (log CFU/g)[a,b]

Storage time	U	G	F	C	S
Initial	$2.41 \pm 0.014_{(a,A)}$	$2.41 \pm 0.007_{(a,A)}$	$2.41 \pm 0.028_{(a,A)}$	$2.41 \pm 0.099_{(a,A)}$	$2.41 \pm 0.021_{(a,A)}$
6 months	$2.41 \pm 0.071_{(a,A)}$	$2.42 \pm 0.057_{(a,A)}$	$2.42 \pm 0.007_{(a,A)}$	$2.42 \pm 0.028_{(a,A)}$	$2.41 \pm 0.127_{(a,A)}$

[a] (U) Macaroni made from 20% unprocessed cowpea flour; (G) macaroni made from 20% germinated cowpea flour; (F) macaroni made from 20% fermented cowpea flour; (C) macaroni made from 20% cooked cowpea flour; (S) macaroni made from 100% durum wheat semolina.
[b] Figures in the same column or row sharing a common letter in parentheses are not significantly different at the 0.05 level.

Table 2
Mold and yeast counts of the macaroni samples enriched with 20 % cowpea flour (log of CFU/g)[a,b]

Storage time	U	G	F	C	S
Initial	$1.84 \pm 0.014_{(a,A)}$	$1.85 \pm 0.007_{(a,B)}$	$1.85 \pm 0.007_{(a,B)}$	$1.84 \pm 0.004_{(a,A)}$	$1.79 \pm 0.016_{(b,B)}$
6 months	$1.86 \pm 0.002_{(b,A)}$	$1.88 \pm 0.003_{(a,A)}$	$1.89 \pm 0.014_{(a,A)}$	$1.85 \pm 0.008_{(b,A)}$	$1.82 \pm 0.014_{(c,A)}$

[a] See Table 1 for abbreviations.
[b] Figures in the same column or row sharing a common letter in parentheses are not significantly different at the 0.05 level.

Table 3
In vitro protein digestibilities of macaroni samples enriched with 20% cowpea flour (%)[a,b]

Storage time	U	G	F	C	S
Initial	$78.5 \pm 0.57_{(a,A)}$	$79.1 \pm 0.50_{(a,A)}$	$78.3 \pm 0.94_{(a,A)}$	$79.4 \pm 0.82_{(a,A)}$	$78.9 \pm 0.99_{(a,A)}$
6 months	$79.3 \pm 0.88_{(a,A)}$	$80.0 \pm 0.74_{(a,A)}$	$78.9 \pm 0.88_{(a,A)}$	$80.2 \pm 0.96_{(a,A)}$	$79.4 \pm 0.44_{(a,A)}$

[a] See Table 1 for abbreviations.
[b] Figures in the same column or row sharing a common letter in parentheses are not significantly different at the 0.05 level.

Giami, Adindu, Hart, and Denenu (2001) found that boiling of African breadfruit seeds for 5–20 min improved protein digestibility.

3.4. Differential scanning calorimetry (DSC)

Results revealed that there were two endothermic transitions during DSC analysis of the samples (Tables 4 and 5). It has been reported that the first endothermic transition relates to the gelatinization of starch in excess water and the second one to the possible denaturation of proteins and reversible dissociation of amylose–lipid complexes (Lupano & Gonzales, 1999; Zweifel, Conde-Petit, & Escher, 2000). The T_{o1} (the onset temperature of the first peak) values obtained in our study were lower than the values of T_{o1} reported for pure cowpea starches (Henshaw et al., 2003). The difference may be due to variations between starches in samples. There were slight but statistically significant differences ($p < 0.05$) between the gelatinization onset temperatures of the samples, ranging from 59.9 to 61.7 °C (Table 4). The processing applied in this study (namely, germination, fermentation, cooking) may cause the differences shown. The mechanisms causing these differences for the onset of gelatinization in the samples during thermal analysis require further research. Small but statistically significant differences were found between the T_{o1} values of samples, whereas the peak temperatures of samples with added processed cowpea were not statistically different ($p < 0.05$) (Table 4). The melting temperature for the sample with unprocessed cowpea flour had a lower melting temperature than the others (Table 4). It is seen from Table 4 that addition of cowpeas to macaroni reduced the enthalpy of gelatinization (ΔH_1) significantly ($p < 0.05$). This may be explained by the increased protein content of samples with cowpea addition, leading to decreased enthalpy values for starch gelatinization (Lundqvist & Eliasson, 2005). A decrease in ΔH_1 values could also mean that the starch in the blend has less available water, due to protein competition (Mohamed & Rayas-Duarte, 2003).

The data for the second peak, during DSC analysis, are given in Table 5. The protein denaturation and dissociation of amylose–lipid complex in the samples started at 78.9–88.9 °C and peaked at 87.6–100.4 °C. The macaroni sample enriched with unprocessed cowpea flour had a peak with the lowest T_{o2} (78.9 °C) and T_{p2} (90.0 °C) with the highest ΔH_2 (enthalpy) (3.86 J/g) among the other samples studied. These values were lower than the values reported for denaturation of cowpea globulins (Henshaw et al., 2003). The differences between the results of different studies may result from different compositions, as well as different sources. The enthalpies obtained in DSC analysis represent a composite, comprising the balance of heat changes involved with gelatinization of starch, denaturation of proteins and the changes associated with protein–starch interactions (Henshaw et al., 2003). Processes such as fermentation and germination are a series of complex reactions which may lead to modifications in many ways. Therefore, the structural modifications occurring during DSC analysis of macaroni enriched with cowpea flour require further research.

Table 4
Thermal properties of macaroni samples enriched with 20% cowpea flour obtained from the first endothermic peak of DSC[a,b]

Sample	T_{o1} (°C)	T_{p1} (°C)	ΔH_1 (J/g)
U	61.7 ± 0.28(a)	66.9 ± 0.14(b)	2.48 ± 0.01(d)
G	59.9 ± 0.14(c)	67.7 ± 0.13(a)	3.37 ± 0.02(c)
F	60.9 ± 0.14(b)	67.9 ± 0.09(a)	2.41 ± 0.02(e)
C	60.5 ± 0.21(b)	67.6 ± 0.14(a)	3.90 ± 0.03(b)
S	60.6 ± 0.14(b)	67.5 ± 0.21(a)	4.21 ± 0.03(a)

Values are for before storage. T_{o1}, onset temperature; T_{p1}, peak temperature; ΔH_1, enthalpy.
[a] See Table 1 for abbreviations.
[b] Figures in the same row sharing a common letter in parentheses are not significantly different at the 0.05 level.

Table 5
Thermal properties of macaroni samples enriched with 20% cowpea flour obtained from the first endothermic peak of DSC[a,b]

Sample	T_{o2} (°C)	T_{p2} (°C)	ΔH_2 (J/g)
U	78.9 ± 0.14(e)	90.0 ± 0.12(c)	3.86 ± 0.08(a)
G	81.2 ± 0.07(d)	86.9 ± 0.15(d)	1.80 ± 0.03(d)
F	82.2 ± 0.01(c)	87.6 ± 0.26(d)	2.79 ± 0.01(b)
C	85.9 ± 0.14(b)	93.8 ± 0.21(b)	2.02 ± 0.01(c)
S	88.9 ± 0.06(a)	100.4 ± 1.55(a)	1.71 ± 0.04(e)

Values are for before storage. T_{o2}, onset temperature; T_{p2}, peak temperature; ΔH_2, enthalpy.
[a] See Table 1 for abbreviations.
[b] Figures in the same raw sharing a common letter in the parantheses are not significantly different at 0.05 level.

4. Conclusion

It can be concluded from the results of this study that addition of cowpea flour at the 20% level did not affect the microbiological quality or in vitro protein digestibility of macaroni samples adversely during a 6-month storage period. Macaroni samples containing cowpea flour had higher enthalpies than the control macaroni with no cowpea added.

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