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Effect of cooking and irradiation on the labile vitamins and antinutrient content of a traditional African sorghum porridge and spinach relish

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Abstract

Irradiation is a potentially useful technology for ensuring the safety and extending the shelf-life of food products in Africa. However, nutritional changes may result. The effects of cooking followed by irradiation (10 kGy) on vitamins B_1 and C, and the antinutritional factors, phytic acid and nitrates, in a ready-to-eat meal of sorghum porridge and spinach-based relish were investigated. Cooking reduced vitamin B_1 and C contents of the spinach relish, and irradiation caused further losses. Cooking did not alter vitamin B_1 content of the sorghum porridge but irradiation decreased it drastically. Cooking did not decrease phytic acid in the sorghum porridge, but irradiation caused a significant decrease. The reduction of antinutritional factors by cooking, followed by irradiation, is promising for the application of this technology to traditional African cereal and leafy vegetable foods. However, ways need to be found to minimise vitamin loss, such as blanching and cooking in minimum water and irradiation at cryogenic temperatures in an oxygen-free atmosphere.

1. Introduction

Cereals and green, leafy vegetables are important components of the diets of African people. Cereals are important providers of food energy (Steller, 1993) and together with leafy vegetables, act as significant sources of macro- and micronutrients (Steller, 1993; Imungi, 1996).

In Southern Africa, cereals, e.g. sorghum and maize, are mostly consumed in the form of porridges (gruels) made from water and meal (Taylor et al., 1997) and generally in combination with a sauce or relish (Bello et al., 1990), which may be prepared from leafy vegetables. An example is sorghum porridge accompanied with a spinach-based relish, which is popular amongst the black population of South Africa. It, however, has a short shelf-life and is laborious to prepare.

Food irradiation is emerging as a major food processing and preservation technology. In Africa, the initial scope of a Co-ordinated Research Programme initiated by the FAO/IAEA is expanding from insect disinfestation of staple foods, to include traditional, processed food products that could be made safe and have extended shelf-life through processing involving irradiation (FAO/IAEA, 1997). Such products could contribute to a more abundant food supply in many areas, especially in those lacking refrigeration (FAO/IAEA, 1997). Further, in Africa which has a high incidence of HIV/AIDS, the provision of irradiated, low microbial load, ready-to-eat foods could be of great benefit to these immunodepressed patients. However, irradiation may cause nutritional losses. Thiamine and ascorbic acid have high sensitivity to irradiation (Kilcast, 1994).

According to Ranum (1997), at the recent International Congress on Nutrition in Montreal, one of the consequences of the 'green revolution' is that the large increase in cereal production, while keeping people supplied with macronutrients, may have actually generated a problem with some micronutrients. This is of concern given the importance of micronutrients in the diet. Ascorbic acid and the B vitamins can affect immune response (Anonymous, 1990). Prophylactic ascorbic acid has been used for upper respiratory viral illness and for combating cold symptoms while B vitamins may act as controlling factors in the rapidly dividing effector cells of the immune system (Anonymous, 1990). AIDS patients experience excessive body

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wasting and major changes in overall body composition indicating, among many problems, depletion of body minerals and vitamins (Reaidi and Cossette, 1992). Antioxidant nutrients, particularly vitamins E, C and β-carotene, continue to occupy a very central position in nutrition research (Anonymous, 1995). This interest stems from scientific curiosity about the mechanism of action of these nutrients and the realisation of the immense potential benefits that these nutrients may have on the health of the general public (Anonymous, 1995).

Anti-nutritional factors, such as phytic acid in cereals and nitrates in leafy vegetables, are of major concern. Phytic acid is said to chelate mineral cations and proteins, forming insoluble precipitates, which leads to reduced bioavailability of trace minerals and reduced digestibility of proteins (Ryden and Selvendran, 1993). Nitrates are precursors of nitrites which react with amines from protein sources to form N-nitroso compounds or nitrosamines which are known to have carcinogenic and mutagenic properties (Mondy and Koushik, 1990).

The objective of this work was to investigate the quality of an irradiated, ready-to-eat meal consisting of sorghum endosperm meal porridge and spinach-based relish with regard to some of its important nutritional and anti-nutritional aspects.

2. Materials and methods

2.1. Materials

2.1.1. Spinach relish

The relish was prepared according to a recipe provided by Mrs R Mathibe, a Tswana speaking South African. Leaves were separated from the stalks of 8 kg fresh spinach obtained from a supermarket. The separated leaves (4 kg) were rinsed in tap water to remove dirt and soil particles and chopped up with a knife into pieces 0.5–1 cm^2. Two cans of tomato and onion mix (Farmgirl brand, Maxim Packers, South Africa) (approx. 1 kg) were added to the leaves, together with 0.6 g salt and then mixed thoroughly with a ladle to obtain the raw relish; which was cooked with gentle heat for 20 min with occasional stirring. Raw and cooked relish was freeze-dried then milled using a pin mill (Retsch, Haan, Germany), fitted with a 750 μm sieve to obtain a fine powder.

2.1.2. Sorghum porridge

Porridge was also prepared according to a recipe provided by Mrs Mathibe. Water (250 ml) was brought to the boil in a pan. Sorghum endosperm meal, 'mabela' (Nola, Randfontein, South Africa) (100 g) was mixed with 50 ml water to form a paste. This was poured into the boiling water and the mixture stirred continuously to prevent lump formation and left to boil for 10 min. Sorghum meal (approx. 400 g) was added in 50 g portions with continuous stirring until the desired consistency was achieved. With the pan covered, the porridge was left to cook for a further 15 min with gentle stirring. The cooked porridge was freeze-dried then milled with a laboratory hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 800 μm screen, to obtain a fine powder.

2.1.3. Irradiated products

Sorghum porridge and cooked spinach relish were dished out into polystyrene trays (16 × 21 cm). The trays were put into full barrier polyethylene bags (polydivinyldichloride-coated polyester; 15 μm barrier abuse layer laminated with 50 μm linear low density polyethylene) and sealed without vacuum using a benchtop vacuum sealing machine. These were then irradiated at a dose of 10 kGy (0.5 kGy h^{-1} for 20 h) using a ^{60}Co source at ambient temperature. Irradiated sorghum porridge and spinach relish were freeze-dried separately and milled.

Sensory evaluation of the product irradiated with doses of 2–10 kGy showed no particular preference for any particular dose. An irradiation dose of 10 kGy is the maximum allowed dose for food commodities without the need for toxicological testing (Thakur and Singh, 1994). Hence this dose was chosen to investigate the maximum effect irradiation could have on the food components and, more especially, microorganisms.

2.2. Methods

All analyses were done on freeze-dried samples.

2.2.1. Vitamin B_1

Vitamin B_1 analysis was done in duplicate by HPLC using a procedure based on that described by Augustin (1994).

2.2.1.1. Sample extraction. Approx 5 g sample was autoclaved at 121°C for 60 min in 60 ml 0.4 M H_2SO_4. After cooling to 45°C, 10 ml 2.5 M sodium acetate was added followed by 100 mg Taka diastase enzyme (Seravac, Cape Town, South Africa). This mixture was incubated at 45°C for 3 h and cooled to room temperature; the volume of each extract was adjusted to 100 ml with distilled water and filtered into brown glass bottles using Whatman No. 2 filter paper, diameter 18.5 cm. Extracts were then stored at −32°C.

2.2.1.2. Thiamine standards. A 1 μg ml^{-1} dilution was prepared from thiamine stock solution (100 μg ml^{-1}) prepared in 0.4 M H_2SO_4 using thiamine hydrochloride (Sigma T4625). Of this solution, 5, 10, 20, 50, 100, 200, and 400 μl were taken; 2 ml 20% KCl/methanol (70/30) solution were added and made up to 5 ml with metha-

nol/H_2O (25/75). Approx 1.5 ml alkaline ferricyanide solution (approx 0.6 ml 5% K_3 $Fe(CN)_6$ made up to 100 ml with 15% NaOH) were added, followed immediately by 0.4 g citric acid crystals. These were then mixed using a vortex mixer and filtered through a 0.45 μm filter.

2.2.1.3. Sample purification. Cartridges (SCX Bond-Elut, 3 cm^3, SMM Instruments, Midrand, South Africa) were washed with 2 volumes of methanol followed by 5 volumes of distilled water. Exactly 2 ml sample extract were applied to each cartridge and, for spiked samples, 2 ml sample extract plus 0.5 ml 1 μg ml^{-1} thiamine standard was applied. Cartridges were then washed with 5 volumes of 5% acetic acid and eluted with 10 ml KCl/methanol (70/30) solution.

2.2.1.4. Conversion to thiochrome. To 2 ml KCl/methanol cartridge effluent, methanol/H_2O (25/75) were added to a total volume of 5 ml. Then 1.5 ml alkaline ferricyanide followed immediately by 0.4 g citric acid crystals, were added. This mixture was then mixed on a vortex mixer and filtered through a 0.45 μm filter and the filtrate was used for HPLC.

2.2.1.5. Reversed phase HPLC. Exactly 30 μl of the filtrate were applied to a Beckman Ultrasphere reversed phase column (4.6 mm×25 cm, 5 μm) and developed with a methanol/0.01 M citric acid buffer (35/65), pH 7.0 mobile phase at a flow rate of 1 ml min^{-1}. A Perkin–Elmer 650 fluorescence detector was used (excitation 360 nm, emission 440 nm, slit 15 nm).

2.2.2. Ascorbic acid

The 2,6-dichlorophenolindophenol visual titration method (Association of Official Analytical Chemists, AOAC, 1980) was used for analysis of ascorbic acid in triplicate.

2.2.3. Phytic acid

The method of Garcia-Villanova et al. (1982) was used for phytic acid analysis in triplicate with slight modifications. After phytic acid extraction from sorghum samples with 0.4 M HCl and 5% sodium sulphate as described in the method, complexometry was done with 0.02 M iron (III) chloride instead of Mohr's salt (ammonium ferrous sulphate) oxidised with ammonium persulphate, and 20% sulphosalicylic acid solution. Back titration with 0.01 M EDTA was done to determine excess Fe^{3+} ions.

2.2.3.1. Phytic acid standards. Phytic acid stock solution (0.01 M) was prepared using 40% w/w phytic acid solution (Cat. No. 28,966-3; Sigma-Aldrich). Phytic acid solutions of different concentrations were prepared by taking, respectively, 1, 1.36, 2, 4, and 8 ml 0.01 M phytic acid. Each of these was made up to 20 ml with distilled water, complexed with sulphosalicylic acid and titrated with EDTA as described above. From blank (20 ml distilled water) and standard titrations, the amount of Fe (III) used was calculated and a calibration graph plotted to determine amount of phytic acid in the samples.

2.2.4. Nitrate

Nitrate was determined (in duplicate) by taking the difference between nitrogen content as measured by the Kjeldahl method and that measured by the Dumas method and converting to nitrate content. The acid digestion process in the Kjeldahl method converts nitrogen in the sample, other than nitrate and nitrite nitrogen, into ammonium sulphate (James, 1995). Nitro, azo and azoxy groups are likely to yield the element or various nitrogen oxides which are lost from the acidic medium (Skoog et al., 1996). The Dumas method proceeds by thermal combustion utilising temperatures in the order of 850–900°C. It is expected that the Dumas method would measure all nitrogen (including nitrate nitrogen) because of its relative severity. Therefore, the difference between the two methods would give an estimate of the nitrate content.

Nitrogen content by the Kjeldahl method was done as described by Chang (1994) using a copper-and selenium-based catalyst.

Nitrogen content by the Dumas method was done using the Leco FP-2000. Approximately 1 g samples were used.

2.2.5. Statistical analyses

Analysis of variance (Statistica, Version 5.0) and the least significant difference test (LSD test) were used to determine whether a difference existed ($p < 0.05$) between means of treatments.

3. Results and discussion

Table 1 shows the effect of cooking and cooking followed by irradiation on the vitamin B_1 content of the spinach-based relish and sorghum endosperm meal. For raw spinach relish, the thiamine content obtained was less than that reported previously (Fordham, 1993) (0.07 mg 100 g^{-1} raw flesh). This may be attributed to difference in cultivar and growing conditions. The thiamine content of raw sorghum meal was less than that reported by Klopfenstein and Hoseney (1995) (0.30 mg 100 g^{-1} at 12% moisture) for whole grains. However, this is to be expected because the sorghum endosperm meal used in this work was decorticated. In sorghum, the B vitamins are concentrated in the aleurone layer and germ and so removal of these tissues by decortication reduces their amount (Serna-Saldivar and Rooney, 1995).

Cooking reduced the vitamin B_1 content of the spinach-based relish significantly on both dry and as-is

Table 1
Effect of cooking and cooking followed by irradiation on vitamin B_1 in spinach-based relish and sorghum endosperm meal

	Vitamin B_1 (mg 100 g^{-1}) Spinach-based relish		Vitamin B_1 (mg 100 g^{-1}) Sorghum endosperm meal	
	Dry	As-is	Dry	As-is
Raw	0.45 a[a] (0.02)[b]	0.03 a (0.00)	0.28 a (0.00)	0.24 a (0.00)
Cooked	0.29 b (0.01)	0.02 b (0.00)	0.28 a (0.01)	0.07 b (0.00)
Cooked and irradiated	0.08 c (0.01)	0.01 c (0.00)	0.04 b (0.00)	0.01 c (0.00)

[a] Mean values in the same column with different letters differ significantly from each other ($p < 0.05$).
[b] Standard error is given in parentheses.

basis. This water-soluble vitamin probably leached out during the blanching process into the blanch water which was discarded, a common cause of vitamin B_1 loss (Tannenbaum et al., 1985). Some chemical degradation during further cooking of the spinach might also have occurred. Whilst cooking reduced vitamin B_1 in the sorghum endosperm meal significantly on an as-is basis, there was no significant effect (increase or decrease) on a dry basis. The decrease on an as-is basis is due to dilution effects. The cooked sorghum porridge contained 76.6% moisture, whereas the raw sorghum contained only 12%. The excellent retention of the vitamin in the porridge on cooking may be attributed to the fact that no effluent was produced during cooking, which rules out the possibility of the vitamin leaching out. Furthermore, in food matrices, some components, such as proteins, are known to protect thiamine, though the protective mechanism is not clear (Dwiveldi and Arnold, 1973).

Irradiation caused a dramatic decrease of thiamine in both components of the meal both on a dry and on an as-is basis. This demonstrates the extreme sensitivity of the vitamin to irradiation as has been noted by other workers (Diehl et al., 1991; Kilcast, 1994). As the products were irradiated in the presence of moisture and oxygen, losses can be attributed to formation of highly reactive primary free radicals from the radiolytic products of water and perhaps secondary reaction products such as hydroperoxides which may have attacked the vitamin (Diehl, 1981).

Table 2 shows the effect of cooking and cooking followed by irradiation on the ascorbic acid content of the spinach-based relish. Literature values for the ascorbic acid (AA) content of spinach vary widely. The 14 mg 100 g^{-1} obtained in this work falls within the wide range reported. Values as low as 10 mg 100 g^{-1} (as-is basis) (Langenhoven et al., 1991) and as high as 52.4 mg 100 g^{-1} (as-is basis) (Vanderslice et al., 1990) have been reported.

Table 2
Effect of cooking and cooking followed by irradiation on ascorbic acid in spinach-based relish

	Ascorbic acid (mg 100 g^{-1})	
	Dry	As-is
Raw	178.8 a[a] (10.6)[b]	14.0 a (2.6)
Cooked	26.7 b (2.7)	2.2 b (0.3)
Cooked and irradiated	18.4 c (1.6)	1.5 c (0.1)

[a] Mean values in the same column with different letters differ significantly from each other ($p < 0.05$).
[b] Standard error is given in parentheses.

Cooking decreased ascorbic acid (AA) significantly on both a dry and as-is basis. This may be attributed primarily to leaching during blanching because of its high water solubility. Several authors have cited similar losses (Sood and Bhat, 1974; Rumm-Kreuter and Demmel, 1990; Vanderslice et al., 1990). During the actual cooking, degradation of the vitamin to diketogulonic acid and further degradation products might have occurred leading to further losses (Gregory, 1985).

Irradiation caused a further decrease of ascorbic acid on a dry and as-is basis. Irradiation can cause a partial conversion of AA to dehydroascorbic acid (Kilcast, 1994) which could account for the losses of AA observed. It is also possible that some degradation of the vitamin could have occurred through its attack by free radicals.

The vitamins studied were chosen, based on their relatively high labilities, to be used as markers to provide an indication of the severity of the processing methods. From the results, it may be speculated that other water-soluble vitamins could be similarly reduced during blanching and cooking. Irradiation may have also degraded other vitamins but not necessarily to the same extent. In comparison to thiamine and ascorbic acid, other B vitamins have low sensitivity to irradiation (Kilcast, 1994).

Table 3 shows the effect of cooking and cooking followed by irradiation on phytic acid content of the sorghum endosperm meal. The quantity of phytic acid in cereal products depends to a large extent on the milling process and the extent to which bran and germ are separated from the endosperm (Hulse et al., 1980). Cereal endosperm usually contains very small amounts (Ryden and Selvendran, 1993). Values ranging between 0.1 and 0.7% have been reported for various sorghum cultivars (Hulse et al., 1980). The phytic acid content of raw sorghum endosperm meal obtained in Table 3 falls within the above mentioned range.

Whilst cooking did not cause a significant decrease in phytic acid on a dry basis, it did so on an as-is basis.

Table 3
Effect of cooking and cooking followed by irradiation on phytic acid content of sorghum endosperm meal

	Phytic acid (mg 100 g^{-1})	
	Dry	As-is
Raw	135 a[a] (16.4)[b]	112 a (12.8)
Cooked	127 a (14.6)	31.5 b (3.8)
Cooked and irradiated	80.5 b (21.0)	18.7 c (4.9)

[a] Mean values in the same column with different letters differ significantly from each other ($p < 0.05$).
[b] Standard error is given in parentheses.

Table 4
Effect of cooking and cooking followed by irradiation on the nitrate content of spinach-based relish

	Nitrate (g 100 g^{-1})	
	Dry	As-is
Raw	1.17 a[a] (0.13)[b]	0.08 a (0.01)
Cooked	0.25 b (0.01)	0.02 b (0.00)
Cooked and irradiated	0.56 b (0.17)	0.04 b (0.01)

[a] Mean values in the same column with different letters differ significantly from each other ($P < 0.05$).
[b] Standard error is given in parentheses.

The stability of phytic acid during normal cooking procedures, which do not involve fermentation has been observed by De Boland et al. (1975) in an investigation of phytate levels in some cereal grains and oilseed products. During cooking, endogenous phytases are deactivated by heat (Ryden and Selvendran, 1993). They are therefore unavailable to break down phytate which can then only be degraded by high temperature processing as employed in the production of cereal-based foods such as cereal flakes (Plaami and Kumpulainen, 1995). Such high temperatures were not used in the preparation of the porridge in this work. The significant decrease in phytic acid due to cooking on an as-is basis was due to dilution effects.

Irradiation of the cooked sorghum endosperm meal decreased phytic acid significantly on both a dry and as-is basis. Reduction of phytate by irradiation has been reported from studies on soybean (Sattar et al., 1990). This reduction may be attributed to chemical degradation of the phytate molecule. The irradiation process may have broken down phytic acid to the lower inositol phosphates and inositol (by the action of free radicals) which have higher solubility and lower chelating power (De Boland et al., 1975). Another possible mode of phytate loss could have been through cleavage of the phytate ring itself.

Table 4 shows the effect of cooking and cooking followed by irradiation on the nitrate content of the spinach-based relish. Nitrate levels in vegetables vary considerably according to species, variety and growing conditions (Bednar et al., 1991). Values ranging from 0.255 to 1.570 g 100 g^{-1} (dry basis) have been reported by different authors (Bednar et al., 1991; Consalter et al., 1992; Forlani et al., 1997). The value obtained for spinach relish (1.17 g 100 g^{-1}) falls within this range.

On both a dry and as-is basis, cooking caused a significant decrease in the nitrate content. This may be attributed to leaching during the blanching operation because of its very high water solubility. Similar losses have been reported by other authors (Bengtsson, 1969; Bednar et al., 1991; Consalter et al., 1992; Forlani et al., 1997).

Irradiation of cooked samples did not cause a significant increase in nitrate content. This is in contrast to what was observed by Mondy et al. (1992) in potatoes. They reported that an irradiation dose of 1.0 kGy resulted in an increase in nitrate-nitrogen concentration of potato tubers with some values 300% greater than controls. During irradiation in the presence of oxygen, highly oxidising free radicals may be formed (Thakur and Singh, 1994) and these may oxidise nitrogen from sources such as proteins and nucleic acids leading to an increase in nitrates. However, components of the spinach relish such as ascorbic acid and β-carotene, known to have antioxidant properties (Francis, 1985; Nawar, 1985), may have exerted a protective effect by reacting with these oxidising radicals.

4. Conclusions

Vitamin losses in the spinach component on blanching and cooking, and further dramatic losses in both components on irradiation, reduced nutritional quality of the sorghum porridge and spinach relish meal. Possible ways of improving vitamin retention could be blanching with less water and in a single change to minimise leaching, irradiation at cryogenic temperatures and in an oxygen-free atmosphere. At low temperature, the mobility of the radicals is reduced, and this results in much slower interactions (Thakur and Singh, 1994). Irradiation in the absence of oxygen has been shown to lead to reduced levels of hydroperoxides and less degradation of vitamins (Thakur and Singh, 1994).

The dilution of nutrients in the sorghum endosperm meal on processing into porridge may be alleviated by using the least amount of water possible without sacrificing palatability.

The reduction of antinutritional factors by cooking, followed by irradiation (nitrates on blanching and cooking and phytic acid on irradiation) is promising for the application of this technology to traditional African cereal and leafy vegetable foods.

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