

Full Length Research Paper

Triple simultaneous stabilizing action of rosemary spice (*Rosemarinum officinalis* L.) in full-fat soya based flour rich in protein and β -carotene

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This study aimed at demonstrating the stabilizing effect of up to 0.4% (wt/wt flour) of rosemary spice (*Rosemarinum Officinalis* L.) in a flour-based protein-rich product intended for young children. The flour was made of full-fat soya flour, meat (beef) powder, and carrot flour as β -carotene source. Analysis was done for proximate composition, β -carotene content, microbial load and sensory evaluation for rancidity over time. Different levels of rosemary spice salvaged a net of 3.42 - 3.83 mg/100 g of β -carotene within a storage period of 7 weeks at 35°C accounting for up to 18% of β -carotene sparing as compared to the non-spicy sample. There was, however, no evidence of increased protection of β -carotene with increase in rosemary spice concentration. Rancid odors and flavour were detected in samples with spice, latter than in samples with no spice. Rosemary spice exhibited up to a net of 38% reduction in microbial load in spicy samples as compared to the non-spicy sample. In a protein, fat and β -carotene rich flour-based product, rosemary spice exhibits triple stabilizing action. The phenolic compounds (rosemarinic, carnosol and carnosic acid) in rosemary spice limits β -carotene degradation and decelerates the production of secondary products of lipid oxidation while the terpene fractions are implicated for halting the proliferation of micro-organisms.

Key words: Rosemary spice, high protein β -carotene-rich flour, antioxidant effect, antimicrobial effect.

INTRODUCTION

It is recognized that poor growth in children results not only from deficiency of protein and energy, but also from inadequate intake of vital minerals (such as iron, iodine and zinc) and vitamins (such as vitamin A), and often fatty acids (UNICEF, 1998). Traditionally, most food preparations for complementary feeding and nutritional rehabilitation are in form of starchy porridges. In Kenya for instance, these foods have been based on maize flour as the major ingredient. GOK and UNICEF (1999) report that about half of Kenyans use porridge as the initial complementary food. The porridge is mostly high in energy but the protein and micronutrient levels are low. This reality has stimulated efforts to increase the concentration of these nutrients in complementary foods using simple and affordable methods.

In sub-Saharan Africa, blending of a food rich in vitamin A with another not so rich in the vitamin (food-to-food for-

tification) has been shown to be a feasible approach to increase the amount of the vitamin in common complementary foods. Incorporation of dried ground carrots as a source of pro-vitamin A in a full fat soya and meat (beef) composite flour was the essence of this research. The product was thus rich in protein, pro-vitamin A (β -carotene) and fats. This made it highly susceptible to microbial spoilage and lipid (and β -carotene) oxidation. Antimicrobial and antioxidative potency of rosemary spice (*Rosemarinus officinalis*) was thus used and its effect on the product investigated. In resource poor settings in the tropics, oxidation of β -carotene (and lipids) and microbial spoilage are quality and safety concerns that limit food marketing and utilization. Technologies such as extrusion (for instance) to increase shelf-stability of oil rich products are normally also not within the rich of micro- and small-scale entrepreneurs in most African settings. There is also increasing trend in food processing of use of natural preservatives. Some chemical additives have been known to have significant health implications. For instance butylated hydroxyanisole (BHA) and butylated hy-

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droxytoluene (BHT) are potent antioxidants but have been found to be carcinogenic (Musa, 2003). Adherent chemical antimicrobials are also widely used but rarely do we have completely safe ones. For instance, Sodium metabisulphite is recognized as a potential cause of air-way irritation and possibly occupational asthma (Steiner et al., 2008). It is against this backdrop that spice (and other natural stabilizers) use has gained currency.

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Shelef, 1983; Zaika, 1988). The antimicrobial potency of rosemary spice has been demonstrated in culture media (Shelef et al., 1980) as well as in foods (Shelef et al., 1984; Kissinger and Zaika, 1978). Rosemary spice is bacteriostatic at 0.3% and bactericidal at 0.5% (Davidson, 1993), just above 0.4% which is the organoleptic threshold, beyond which it is generally unacceptable (Oiyee and Muroki, 2002). Antioxidant properties of rosemary spice in foods have also been investigated (Musa, 2003) albeit most of them in oil products depicting antioxidant potency of rosemary spice. Further, there is paucity of research that attempt to study the potential concurrent benefits of rosemary spice use in foods.

This study aimed at demonstrating the simultaneous triple stabilizing action of utilizing rosemary spice in a flour-based product. That is its propensity to limit β -carotene degradation, slow down production of secondary products of oxidation of fat and decelerate microbial growth.

METHODS

Product formulation and basis

A formulation of full-fat soya flour, meat (beef) powder and carrot flour was made with some considerations in mind. First, Legumes are generally deficient in sulphur-based amino acids, namely cysteine and methionine. Meat (beef), which is rich in these amino acids was thus included to make the amino acid profile of the product complete. Animal source foods, beef included, have also been implicated for positive outcomes in physical and cognitive development of children. Since the formulation was meant for the pre-school children (1 - 5 years old), the addition of beef to the formulation was thus justified. It was targeted that the product would have at least double the protein content of maize-based porridge when added to it in small quantities (1 - 5 g). By computation, a minimum of 64% soya flour and small amount of dried beef (7%) meets this target. The product was designed to provide not less than 60% of the recommended daily allowance (RDA) of vitamin A for 1 - 5 years old children as reported by Sehmi (1993) when added in small quantities (1 - 5 g) to 100 g porridge containing 7% maize flour. This was with the assumption that young children are fed with 400 g of maize flour per day, mostly in the form of maize-based porridge. By computation, the developed flour could provide 60% of vitamin A RDA only when it was composed of about 29% carrots. Rosemary spice was added at organoleptically acceptable levels (0.1 - 0.4%) as reported by Oiyee and Muroki (2002). The computations for the

formulation were done using the food composition tables (of Kenya) developed by Sehmi (1993).

Processing methods

The soya beans were sorted and washed, soaked overnight in water (ratio 1:2, soya to water). They were dehusked by hand and cooked for 30 min (in 2 L of water per 1 kg of dry soya bean) before solar drying (to about 5% moisture content) and milling. Soaking, dehusking and heating were done to reduce the levels of antinutrients. Raw beef was washed, defatted manually then minced and dried in air circulated oven at 80°C before milling. Carrots were sorted and washed before chopping to about 2 mm thick rings. The rings were then blanched for 2 min in boiling water then solar dried and milled. Commercially dried and packed rosemary spice was used in the study. Ingredients in the formulations were same except for the concentrations of rosemary spice.

Proximate and β -carotene analysis

The proximate composition and β -carotene content of the soya flour, dried beef and dried carrots mixtures were determined according to AOAC methods (AOAC, 1984). Sample preparation for β -carotene analysis was done as follows. After processing, the samples were packed in sealed brown opaque paper bags and stored at two temperatures; 4°C, refrigeration temperature and 35°C, the highest average temperature in Kenya. Storage at 4°C was done in the attempt to demonstrate if lower temperature would have an effect on β -carotene change. The flour mixtures were sampled for β -carotene analysis immediately after processing and after every 2 weeks for 7 consecutive weeks.

Rancidity determination by sensory evaluation

Mounts and Warner (1980) have described the procedures for sensory evaluation of soya oil that have been found most useful. These procedures were adopted for the purpose of testing the odour and taste (flavour) changes due to autoxidation of the lipids in the soya-based flours. The flour mixtures were packed separately in sealed brown paper bags and kept at 55°C. 24 h storage at 55°C (accelerated storage temperature) was equaled to 1 month at ambient temperature.

Eleven healthy randomly selected adult volunteers from the department of Food Technology and Nutrition, Faculty of Agriculture, University of Nairobi, Kenya, were screened for sensory evaluation. After training on the sensory evaluation procedure, a triangle test was performed by randomly providing the panelists with one obviously rancid flour and two fresh samples. They were then instructed to single out (by sniffing and tasting) one sample that differed from the rest and in which way it differed. Six panelists correctly singled out the rancid sample.

However, one panelist (out of the six) who was pregnant was excluded, leaving eligible panelist number to five. The five were trained on the procedures of rancidity evaluation (odour and flavour testing). The rancidity test was conducted at an interval of 2 days until either the rancid flavour and odour was detected in at least 3 samples provided to the panelists.

For odour testing, the samples were labeled as R00 to R04, put in covered containers and randomized for odour testing. Covers of the evaluation containers were removed as the evaluators sniffed the volatiles. The panelists recorded the type and intensity of rancid odour in the rating of three; 0 for no odour, 1 for low, 2 for medium and 3 for high odour intensity. Fresh samples were expected not to exhibit detectable rancid odour. For flavour testing, the samples were arranged from left to right starting with one suspected to

Table 1. Nutrient composition of high protein vitamin A-rich flour.

Nutrient	Content in grams per 100 g flour*
Moisture	3.80 ± 0.08
Protein	37.70 ± 0.61
Lipids	16.60 ± 0.00
Carbohydrates	29.00 ± 0.64
Ash	2.50 ± 0.03
Fibre	10.50 ± 0.02
β-carotene (in mg per 100 g flour)	22.50 ± 0.19

*± Confidence interval (CI)

to have weakest rancid flavor; R04 through R00. Each panelist took about 5 g of the flour into the mouth and thoroughly swished. Tap water boiled and cooled to 38°C was used to rinse the mouth before tasting, between each sample and after completion of the exercise. The panellists re-corded the type and intensity of rancid flavour in the rating of three; 0 for no flavour, 1 for low, 2 for medium and 3 for high flavour intensity. Fresh samples were expected not to exhibit detectable rancid flavour.

The means of the scores recorded by the panellists were calculated and tabulated. The rancid odour and flavour was considered present when it was in overall, at least slightly detectable (mean score was equal or more than 1).

Microbial analysis (Total plate count)

Samples were stored at 35°C and at refrigeration temperature (4°C). Total plate count was done on weeks 2, 5 and 7 for samples stored at 35°C and only week 7 for samples stored at 4°C. 20 g from each treatment was sampled, weighed out and transferred in to a sterile blend jar (Corydon, England). Four Hundred and fifty milliliters of 0.85% saline solutions were then added and the flours homogenized for 2 min each. Serial dilutions were then made up to dilutions of 10⁻⁷. Dilutions 10⁻⁴ - 10⁻⁷ were plated in the plate count agar and incubated for 48 h after which the colonies with visible growth were enumerated. The trend of microbial growth in all samples was then analyzed.

Statistical analysis

For all the readings the means were reported. β-carotene data was further subjected to the analysis of variance (ANOVA) and post-hoc analysis using Statistical Package for Social Scientists version 11 (SPSS v 11) to compare the differences with changing rosemary spice levels. Post-hoc analysis was done using Duncan multiple range test procedure as described in the SPSS software. A 5% significant level (P > 0.05) was used in all the analyses.

RESULTS

Composition of basic formulation

Five samples formulated had the same composition as shown in Table 1 but varying levels of rosemary spice (0.0 - 0.4% wt/wt flour). The flours had moisture content of 3.8% which is acceptable in Kenya. The protein and the lipid contents were high thus making the product highly susceptible to high rate of microbial growth and lipid oxidation. The β-carotene was 22.5 mg per 100 g of the

flour.

Beta-carotene stability

Over a period of 7 weeks of storage at 35°C, the β-carotene content in all the samples changed as shown in Table 2. At week 0, immediately after processing, the β-carotene content of all the samples were comparable (p>0.05). From week 1 through week 7 of storage at 35°C, sample R00 (containing no rosemary spice) exhibited the lowest and significantly different (p<0.05) β-carotene content as compared to other samples.

At week 1, sample R03 (containing 0.3% rosemary spice) had the highest β-carotene content and differed significantly from the rest of the samples (P<0.05). At week 3, sample R02, with 0.2% rosemary spice had the highest β-carotene content and differed significantly with other samples except for R04, the sample with the highest rosemary spice (0.4%). At week 5, R01 had the highest level of β-carotene and significantly differed with other samples except sample R04. At week 7 at 35°C storage, R04, sample with the highest spice level had the highest level of β-carotene and significantly differed with the rest of the samples.

At 4°C R01 had the highest β-carotene level (at week 7) and significantly differed with other samples. Sample R00, with no spice at all, had the lowest β-carotene content but did compare with sample R02 at the refrigeration temperature. At this storage temperature, β-carotene values at week 7 were slightly higher than those of week 0 but the differences were not significant. This was probably because of experimental errors.

Against the expectations, there was no show, of increases β-carotene protection with change in spice concentration.

In terms of percentage change in β-carotene content, there seemed to be an obvious difference between sample with no spice and other samples. As compared to the control sample, the spice salvaged up to 18% (% change in R04 subtracted from % change in R00) of β-carotene. Another perspective of the analysis (plot of percentage change at different points in time) revealed that the percent loss of β-carotene at any given point in time was far much higher in sample with no spice (Figure 1) as com-

Table 2. Beta-carotene content of flour mixtures stored at 35°C and 4°C over a period of 7 weeks

Samples ¹	β-carotene content in mg/100g						Stored at 4°C Week 7
	Stored at 35°C					% change ²	
	Week 0	Week 1	Week 3	Week 5	Week 7		
R00	22.47 ^a ± 0.26	16.40 ^e ± 0.25	12.60 ^d ± 0.14	9.58 ^c ± 0.10	7.22 ^d ± 0.10	67.87	20.75 ^d ± 0.10
R01	22.21 ^a ± 0.13	19.84 ^c ± 0.07	14.84 ^c ± 0.32	12.87 ^a ± 0.07	10.64 ^c ± 0.07	52.09	24.63 ^a ± 0.06
R02	22.24 ^a ± 0.07	20.34 ^b ± 0.26	17.17 ^a ± 0.07	12.74 ^d ± 0.13	10.87 ^b ± 0.00	51.47	20.83 ^d ± 0.00
R03	22.13 ^a ± 0.78	21.27 ^a ± 0.26	16.50 ^b ± 0.20	12.68 ^b ± 0.10	10.74 ^b ± 0.07	51.47	22.58 ^c ± 0.04
R04	21.97 ^a ± 0.07	19.27 ^d ± 0.39	16.80 ^a ± 0.25	12.72 ^a ± 0.10	11.05 ^a ± 0.10	49.70	23.27 ^b ± 0.13

¹All samples contain 64% of soya flour, 29 % carrot powder and 7% beef powder.

Acronyms: R00 contains no rosemary spice, R01 had 0.1%, R02 had 0.2%, R03 had 0.3% and R04 had 0.4% of the rosemary spice based on weight of the flour mixtures. Values with the same letters in the same column are not significantly different at 5% significant level ($P>0.05$) - Based on Duncan's Multiple Range test.

²The change is between week 7 and week 0.

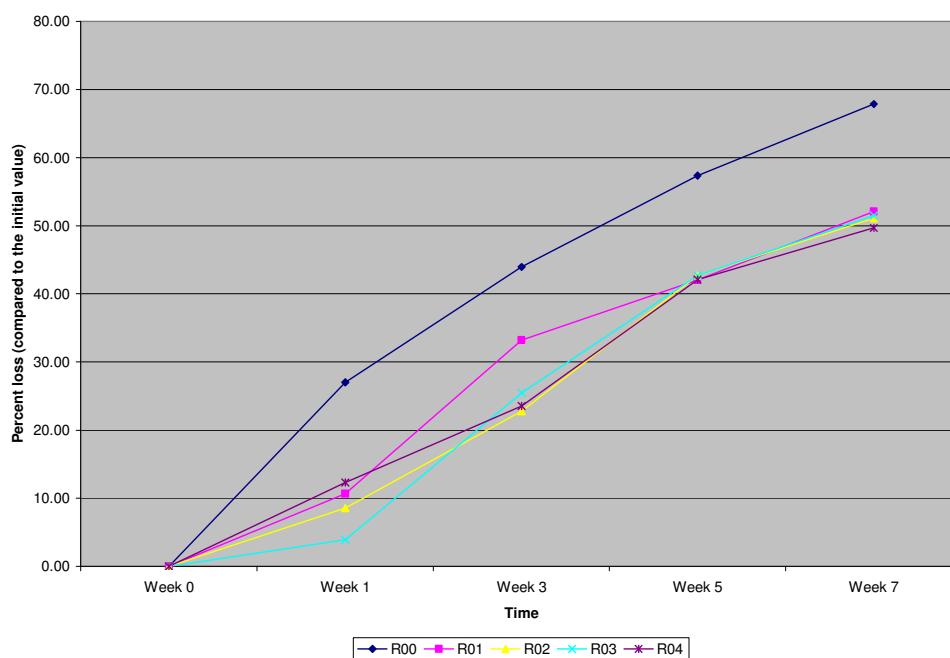


Figure 1. Losses (decreases) of β-carotene between weeks for samples stored at 35°C.

pared to the rest of the samples. It was also observed that in the short run, the potency of different spice levels highly differed (with no definite pattern with concentration). However, in the long run, their propensity to salvage β-carotene seem comparable as depicted by the convergence of percent change in β-carotene content among samples with the spice.

Production of secondary products of oxidation

The mean scores for rancid odours and flavours as detected by 5 trained panelists are shown in Table 3. Rancidity was considered detectable when the mean for the five trained panelists was at least 1.

The rancid odour and flavour in R00 was first to be de-

tected after day 6 and 8 respectively. After the initial detections in R00, the intensities increased with time. These attributes were detected in some samples with rosemary spice at much latter (day 10) - rancid odour in R01 and R03, and rancid flavour R03 and R04. Rancid odour was not detected in samples R02 and R04 by day 10 while rancid flavour went undetected in R01 and R02 within the ten days of storage at accelerated storage temperature equivalent to 10 months.

Microbial growth

As shown in Table 4, sample R00 (without rosemary spice) had microbial load above the maximum recommended in food samples (1.0×10^6 CFU/g) in Kenya 2

Table 3. Mean scores for rancid odours and flavours over time for samples stored at 55°C.

Sample ¹	Mean odour scoring						Mean flavour scoring					
	Time in days ²						Time (days ²)					
	0	2	4	6	8	10	0	2	4	6	8	10
R00	0	0	0	1.2	2.4	2.6	0	0	0	0	1.4	2.8
R01	0	0	0.4	0	0	1.9	0	0	0	0	0	0.8
R02	0	0	0	0	0	0	0	0	0	0	0	0.8
R03	0	0	0	0	0	1.4	0	0	0	0	0	1.4
R04	0	0	0	0	0	0	0	0	0	0	0.4	1.2

¹Acronyms: Sample R00 contains no rosemary spice, R01 had 0.1%, R02 had 0.2%, R03 had 0.3 and R04 had 0.4%.

²One day storage at 55°C (accelerated storage temperature) was equaled to 1 month at ambient temperature

Table 4. Total plate count of flours stored at 35°C and 4°C for 8 weeks.

Sample ¹	Colony Forming Units per gram				
	Stored at 35°C				Stored at 4°C
	Week 2	Week 5	Week 7	% change ²	Week 7
R00	1.1×10^7	7.6×10^6	1.0×10^7	-9.09	7.9×10^6
R01	1.1×10^6	4.4×10^5	5.8×10^5	-47.27	8.0×10^5
R02	1.3×10^6	7.8×10^5	8.5×10^5	-34.62	2.5×10^4
R03	1.1×10^6	1.9×10^5	1.1×10^6	0.00	4.0×10^5
R04	1.1×10^6	5.5×10^5	7.9×10^5	-28.18	8.0×10^4

¹Acronyms: R00 contains no rosemary spice, R01 had 0.1%, R02 had 0.2%, R03 had 0.3 and R04 had 0.4% of the rosemary spice based on weight of the flour.

²The change is between week 7 and week 0.

weeks after processing. Samples with rosemary spice had microbial loads comparable to the maximum recommended load.

In all the samples, there was an observed initial sharp decrease in microbial load at week 5. This initial decrease brought load in the samples to below the maximum allowed except for the spicy sample. For spicy samples, microbial load was maintained below maximum allowed after the initial sharp decline at week 5. At final storage time (week 7), samples stored at 4°C had lower loads than at week 2 and were way below the maximum allowed in foods.

In overall, over a period of 7 weeks at 35°C, there was a depiction of rosemary spice halting effect on the microbial growth but no observation of augmented action with increased concentration. Going by the percent change in the load over a period of 7 weeks, rosemary spice was responsible for a net reduction (compared to the sample without spice) of between 9% (in sample R03) and 38% (in sample R01) of the microbial load.

DISCUSSIONS

The results show that β -carotene is inevitably degraded with or without the natural antioxidant (rosemary spice).

However, addition of rosemary spice at 0.1 - 0.4% resulted in better β -carotene retention - equivalent to up to 18% more retention than without the spice. The antioxidant nature of the spice is attributed to rosmarinic, carnosol and carnosic acid (Allen and Hamilton, 1989). There are indications however, that the antioxidant activity of rosemary extract mainly depends on carnosic acid content (Hudson, 1990). These implicated compounds are all phenolic in nature and they implicated compounds are all phenolic in nature and they do donate hydrogen atoms to capture free radicals and thus halt the oxidation process. The donated hydrogen atoms are depleted and are non-renewable. This explains the reduction of β -carotene levels in samples containing rosemary spice. In other words, the antioxidant potency of the spice gets used up with time. Loss of β -carotene may also not only be as a result of oxidation, but also through stereoisomerisation (Simpson, 1985) which is not halted by the phenolic compounds. At 0.1 - 0.4% rosemary spice levels in composite flour, there is no appreciable improved antioxidant benefit with increase in spice concentration. Heat is a crucial factor in β -carotene degradation. The samples with or without rosemary spice stored under refrigeration for a period of 7 weeks remained relatively stable in terms of β -carotene content. Carotenes are susceptible to

cis-trans inversion at elevated temperatures and oxidation of β -carotene is also heat dependent (Simpson, 1985). Low temperature is a stabilizing factor for β -carotene.

Antioxidant activity of rosemary spice also delays the production of secondary products of fat oxidation as exhibited by the delay in development of rancid odour and flavour. This is attributed to the same phenolic compounds mentioned above, which capture free radicals and halt chain reaction during oxidation of fat by donating the hydrogen atoms. The incorporation of rosemary spice leads to delayed onset of rancid odour and flavour development by up to 4 months.

It was also demonstrated that rosemary spice has inhibitory effect on microbial proliferation in the protein rich flour. Albeit the mechanism is not clear, rosemary inhibitory effects can be attributed to terpene fraction, which is composed of borneol, aneole, pinene and camphor (Jay, 1987). The phenolic compounds have also been implicated (Jay, 1987; Weiser et al., 1971). The inhibitory effects of rosemary spice have been found to be bacteriostatic at 0.3% and bactericidal at 0.5% (Davidson, 1983). Rosemary spice has been found particularly effective against *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* (Shelef et al., 1980). As compared to high temperature, refrigeration halted microbial growth irrespective of the presence or absence of the spice. Sharp decrease in the load at the initial period of storage in all samples can be explained by death of some heat sensitive microbes (heat shock). It also seemed that antimicrobial benefit of rosemary spice in the flour-based product is not dose dependent at organoleptically acceptable concentrations of 0.1 - 0.4%.

Conclusions

The study established that at organoleptically acceptable levels of 0.1 - 0.4%, rosemary spice (*R. officinalis* L.) exhibits triple stabilizing action in a flour mixture of soya bean and beef as protein sources, and carrots as β -carotene source. The phenolic compounds in rosemary spice act to some appreciable extent against β -carotene degradation and rancidity development while the terpene fractions are thought to halt microbial proliferation. Rosemary spice can be used to stabilize flour-based food products. At 0.1 - 0.4% concentration, the stabilizing action of rosemary spice is not dose dependent.

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