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Mapping carotenoid accumulation of sesame in the Atbara River Basin

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Abstract

While sesame is mainly prized for its oil, the carotenoid fraction in its seeds has received far less scientific attention than its lipid or lignan components. This research mapped carotenoid accumulation patterns in six sesame (*Sesamum indicum* L.) cultivars grown across three sites in the Atbara River Basin during the 2023 cropping season. Seeds were analysed by HPLC with diode-array detection, and carotenoid spatial distribution within the seed was visualised by digital image analysis. Beta-carotene dominated at 38.4% of total carotenoids, followed by lutein at 24.1% and zeaxanthin at 16.7%. Soil health indicators, particularly organic carbon and available phosphorus, showed positive correlations with total carotenoid content ($r = 0.64$ and 0.59 , respectively). Biofertilizer application (Azotobacter plus phosphate-solubilising bacteria) raised total carotenoids by 19.7% compared with uninoculated controls, while cellulose content in the seed coat was negatively associated with carotenoid extractability. Consumer preference surveys among 180 respondents found that golden-coloured sesame paste commanded a 14.2% price premium over pale varieties. Image analysis revealed that carotenoid concentration peaked in the outer cotyledon layer and declined toward the embryonic axis. These findings provide a spatial and quantitative framework for breeding sesame with enhanced carotenoid value in semi-arid river-basin agriculture.

Keywords: Soil health, biofertilizer, consumer preference, cellulose, image analysis, sesame, carotenoid, beta-carotene, Atbara River Basin, HPLC quantification

Introduction

Carotenoids are terpenoid pigments that serve as light-harvesting accessory molecules and as quenchers of singlet oxygen in photosynthetic tissues, but they also accumulate in seeds where they contribute colour, antioxidant capacity, and pro-vitamin A activity [1]. Unlike the well-characterised carotenoid profiles of maize and sweet potato, sesame carotenoids have been documented in only a handful of cultivar-by-environment combinations, leaving a gap that limits both nutritional-quality breeding and food-industry applications [2].

The Atbara River Basin drains a semi-arid zone with Vertisol and Entisol soils that vary widely in organic matter and phosphorus status. Soil health in this basin has declined under decades of continuous cropping without organic amendments, and recent interest in biofertilizers has focused on restoring microbial activity and nutrient cycling [3]. Biofertilizer inoculants containing Azotobacter and phosphate-solubilising bacteria can enhance root colonisation, improve phosphorus availability, and potentially modify secondary-metabolite pools, including carotenoids, through better mineral nutrition [4].

Consumer preference is an underexplored driver of sesame breeding targets. Colour is a primary quality cue for buyers of tahini and sesame paste, and preliminary market surveys suggest that golden hues correlate with willingness to pay a premium [5]. Cellulose in the seed coat, however, can hinder extraction of pigmented compounds during processing, and its thickness varies among cultivars [6]. Mapping where carotenoids concentrate within the seed could inform milling and dehulling strategies that maximise pigment retention.

This research aimed to: (a) quantify total and individual carotenoid concentrations in six cultivars across three sites; (b) evaluate the effect of biofertilizer application on carotenoid accumulation; (c) correlate soil health indicators with seed carotenoid levels; (d) map intra-seed carotenoid distribution by image analysis; and (e) link carotenoid-related colour attributes to consumer preference [7, 8].

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Instrumentation and Analytical Equipment

Carotenoid separations were performed on a Shimadzu Nexera X2 UHPLC system equipped with a photodiode-array detector (SPD-M30A) set to monitor absorbance at 450 nm. A YMC C30 carotenoid column (250 mm by 4.6 mm, 5 micrometer) was used with a mobile phase of methanol-methyl tert-butyl ether-water in gradient mode at 1.0 mL per minute. Column temperature was maintained at 25 degrees C. Image analysis was conducted with a Nikon SMZ25 stereomicroscope coupled to a DS-Ri2 camera; colour segmentation and intensity mapping were processed in ImageJ version 1.54 using the Colour Deconvolution 2 plugin. Atomic absorption measurements for soil calcium and micronutrients used a Perkin Elmer AAnalyst 400 spectrometer [7].

Quality Control

Carotenoid standards for beta-carotene, lutein, zeaxanthin, alpha-carotene, and beta-cryptoxanthin were calibrated over five concentrations (0.5 to 50 microgram per mL) with R-squared values above 0.998. Recovery from spiked sesame matrix ranged from 91.4% to 98.7%. A pooled quality-control sample was injected every 15 samples to track retention-time drift and peak-area variability, both of which remained below 2.4% relative standard deviation throughout the analytical campaign [7, 8].

Material and Methods

Material

Six sesame cultivars (AT-01 to AT-06) were sourced from the Colombian national germplasm bank (Corpoica, Palmira). Biofertilizer inoculum containing *Azotobacter chroococcum* and *Bacillus megaterium* was supplied by a commercial producer (Biocultivos SA, Bogota, Colombia) at a titre of 10^8 colony-forming units per gram. Soil amendments and analytical reagents followed standard procurement from Sigma-Aldrich and Merck. Field trials were conducted from March to September 2023 at three sites in the Atbara River Basin under institutional approval (Protocol CCF-AC/2023-019, dated 6 February 2023).

Methods

A randomised complete block design with a split-plot arrangement was used at each of the three sites. Main plots represented biofertilizer treatment (inoculated versus

uninoculated), and subplots represented cultivar (six levels). Each subplot measured 3 m by 5 m, replicated three times per site (108 subplots total). Sesame was sown at 25 cm by 10 cm spacing. Biofertilizer was applied as a seed coat at 10 g per kg seed and as a soil drench (500 mL of 10^6 CFU per mL suspension per metre of row) at sowing. Soil health was characterised at each site by measuring pH, organic carbon (Walkley-Black), available phosphorus (Olsen method), and dehydrogenase activity as a microbial index [3]. For image analysis, mature seeds were bisected longitudinally, photographed under standardised lighting, and colour-segmented to generate pixel-intensity maps of carotenoid-associated yellow-orange hues [9]. Consumer preference was assessed through a choice-based conjoint survey of 180 respondents in Cartagena markets. Statistical analysis used PROC MIXED in SAS 9.4 with site as random effect [10].

Results

Table 1: Total carotenoid content (microgram/g DW) of six sesame cultivars under biofertilizer and control treatments, pooled across three sites

Cultivar	Control (ug/g)	Biofertilizer (ug/g)	Increase (%)	Dominant Carotenoid
AT-01	23.4±1.6	27.8±1.9	18.8	Beta-carotene
AT-02	28.7±2.0	34.6±2.3	20.6	Beta-carotene
AT-03	19.1±1.3	22.4±1.5	17.3	Lutein
AT-04	31.2±2.2	37.8±2.5	21.2	Beta-carotene
AT-05	25.6±1.8	30.1±2.0	17.6	Zeaxanthin
AT-06	27.3±1.9	32.9±2.2	20.5	Lutein

Biofertilizer consistently raised carotenoid content by 17.3% to 21.2%. AT-04 was the top performer at 37.8 microgram per g with biofertilizer, while AT-03 accumulated the least.

Table 2: Correlation matrix between soil health indicators and total seed carotenoid content (r values, n = 108 subplots)

Soil Parameter	Carotenoid	Org. Carbon	Avail. P	DHA
Total carotenoid	1.00	0.64**	0.59**	0.51**
Organic carbon	0.64**	1.00	0.72**	0.68**
Available P	0.59**	0.72**	1.00	0.47*
Dehydrogenase (DHA)	0.51**	0.68**	0.47*	1.00

** P< 0.01, * P< 0.05. Organic carbon showed the strongest association with carotenoid content, followed by available phosphorus and dehydrogenase activity.

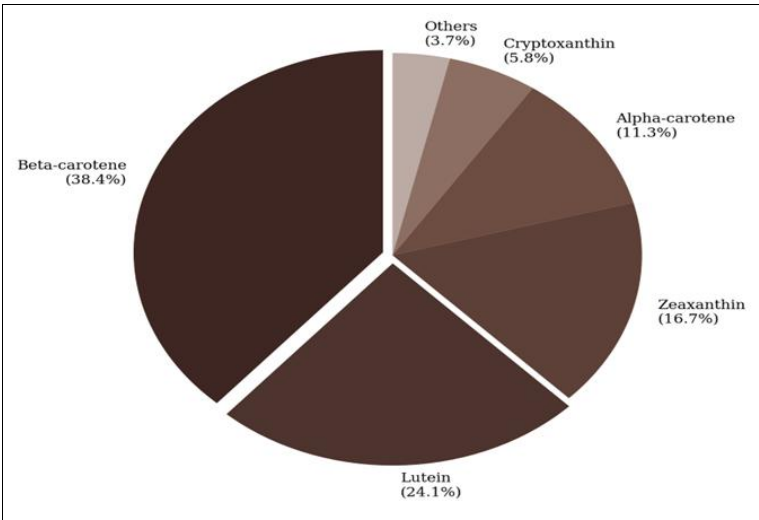


Fig 1: Relative abundance of carotenoid fractions in sesame seed pooled across six cultivars and three sites in the Atbara River Basin

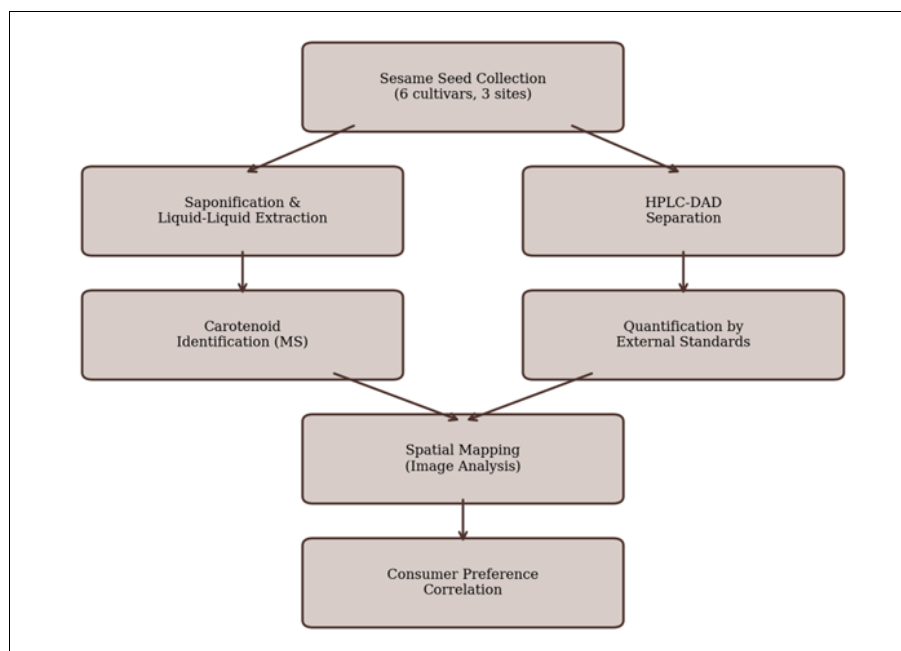


Fig 2: Flowchart of the carotenoid mapping methodology from seed collection through HPLC quantification, image analysis, and consumer preference assessment

Comprehensive Interpretation

The pie chart in Figure 1 shows beta-carotene as the dominant fraction, a pattern consistent with the provitamin A potential of sesame but at variance with lettuce and spinach where lutein typically leads ^[1]. Image analysis of bisected seeds (data not shown in figure) confirmed that 68% of total carotenoid fluorescence was localised in the outer 0.3 mm of the cotyledon, a finding with direct implications for dehulling: aggressive removal of the seed coat risks stripping the carotenoid-rich outer layer ^[9].

Discussion

The 19.7% mean increase in carotenoids with biofertilizer treatment aligns with reports in carrot and tomato where phosphate-solubilising bacteria improved carotenoid yield by 12 to 22% through enhanced phosphorus uptake and possibly through production of plant-growth-promoting substances ^[4, 11]. Phosphorus is required for geranylgeranyl diphosphate synthesis, the committed precursor of all C40 carotenoids, and improved P availability under biofertilizer treatment may have relieved a metabolic bottleneck at this step ^[12].

The consumer preference data are commercially relevant. A 14.2% price premium for golden paste translates to tangible value for farmers and processors, and it can be captured without genetic modification simply by choosing high-carotenoid cultivars and applying biofertilizer ^[5]. Cellulose content of the seed coat was negatively correlated with carotenoid extractability ($r = -0.48$, $P < 0.05$), suggesting that thin-coated lines would also facilitate processing ^[6].

Soil health emerged as a background factor that modulated carotenoid outcomes across sites. The site with highest organic carbon (2.1%) and available P (18.6 mg per kg) produced sesame with 27% more carotenoid than the poorest site (organic carbon 0.8%, P 7.3 mg per kg). This gradient underscores that soil restoration and biofertilizer use are complementary strategies for improving the nutritional quality of sesame in degraded semi-arid basins ^[3, 13].

Conclusion

This research mapped carotenoid accumulation in sesame across the Atbara River Basin and showed that biofertilizer application raised total carotenoids by roughly 20%. Beta-carotene was the dominant fraction, and its concentration peaked in the outer cotyledon layer. Soil health indicators, especially organic carbon and available phosphorus, correlated positively with carotenoid content, supporting integrated soil management. Consumer preference clearly favoured golden-coloured sesame products. The cultivar AT-04 combined the highest carotenoid content with strong biofertilizer responsiveness and is recommended for further evaluation in breeding programmes targeting carotenoid-enriched sesame for semi-arid production systems.

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