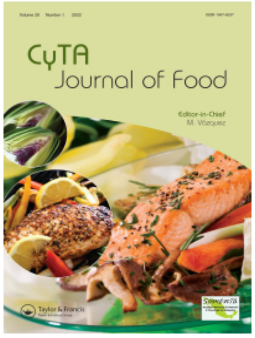

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Effect of processing methods on nutrient and anti-nutrient composition of grasshopper and termites

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ABSTRACT
Different processing methods could impact on the nutritional and anti-nutritional composition of edible insects. This study involved the analysis of nutrients and anti-nutrients in fresh and processed grasshopper (*Ruspolia differens*) and winged termites (*Nasutitermes spp*) in Kenya. Proximate analysis was done using AOAC methods, minerals analysis using AAS and anti-nutrients analysis using UV-VIS and titration. Moisture content reduced significantly to less than 10% on processing, while ash content increased significantly by more than 50% on processing. Processing did not affect the crude fibre content but it led to a 0.2–90% decrease in crude fat. Processing increased significantly the protein content but did not significantly influence mineral levels; however calcium content was reduced considerably. Anti-nutrient levels decreased significantly on processing by 2%–70% with oxalates and phytates having the highest decrease. These results show that oven-drying and defatting methods retained higher nutrient composition in the edible insects.

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Termite; grasshopper; defatting; oven-drying; sun-drying

1. Introduction

Global food insecurity is an immense challenge due to an increasing human population, inadequacies in food production and climate changes (Nwozor et al., 2019). The immediate consequence of food insecurity is malnutrition, with millions of children currently affected by malnutrition worldwide (Bliss et al., 2016). Recent studies have indicated high protein deficiency among Kenyan children (Kinyuru et al., 2018). Animal proteins such as eggs, beef and milk, the primary sources of animal proteins for human consumption, are rated higher in quality than plant proteins (Bryant, 2022). However, these animal proteins are pricey and not readily available (Sahito et al., 2022). Sustainable unconventional sources of proteins, such as edible insects, have been sought (Lange & Nakamura, 2021). Insect consumption is a potential solution to the challenge of the continually increasing food demand (Ebenebe et al., 2017).

Edible insects have been consumed for ages in various parts of the world, such as Asia, Australia, South American countries, the majority of Asian countries and Africa (Van Huis, 2016), with more than 2,000 insect species consumed globally (Roos & Van Huis, 2017). Some edible insects are harvested from their natural habitat, while others are reared. Such insects include grasshoppers, locusts, black soldier flies, lake flies, crickets, and termites (Kinyuru et al., 2018). For instance, in East Africa, edible insects husbandry schemes have been set, and some nations like Kenya have developed guidelines that will govern safe insect rearing and production (Kenya Bureau of Standards [KEBS], 2017).

Edible insects are a suitable source of proteins and minerals which are vital nutrients required in the human diet (Finke, 2015). Their protein levels are comparable to those in animal and plant based proteins (Van Huis, 2016). However, the nutritional content and anti-nutritional content of insects varies strongly with species, geographical location, feed substrates, stage of metamorphosis and processing techniques (Lange & Nakamura, 2021; Meyer-Rochow et al., 2021).

Anti-nutrients such as phytic acid are abundant in various plants and some insects feed on these plants. This increases the concentration of anti-nutrients in the insects (Thakur & Kumar, 2017) and may decrease nutrient bioavailability which can be lethal when they exceed the permissible limits (Samtiya et al., 2020). However, they can easily be detoxified during processing (Samtiya et al., 2020). For this reason, reducing their levels in edible insects is an area of concern owing to the need of preventing toxicity and illness caused by these anti-nutrients (Joye, 2019).

Communities that consume insects have diverse traditional processing techniques such as sun-drying, boiling, roasting and frying (Kinyuru et al., 2018) to make the edible insects tasty (Mutungi et al., 2019). Processing eventually affects the final product regarding nutritional and anti-nutritional composition (Manditsera et al., 2019). For instance, a study on fresh pupae of *Bombyx mori* in India found that the protein content was 17.1 g/100 g protein compared to 56.9–75.2 g/100 g protein of sun-dried pupae (Ademola et al., 2017). However, studies on the effect of thermal treatment and defatting processes on nutrient and anti- nutrient content in edible insects are few. Hence this study was undertaken to determine the quality regarding nutrient and anti-nutrient composition of processed (sun-dried, oven-dried and defatted) grasshopper and termites.

2. Materials and methods

2.1. Sample collection and preparation

The termites (winged termites) were collected from the wild in Bondo sub-county in Siaya County, while the

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grasshoppers (adults) were collected from Khwisero Sub-County in Kakamega County. The insects were put in plastic bags and kept in cool boxes containing dry ice and transported to the Kenyatta University for laboratory analysis within 12 hours of collection. A kilogram of each insect sample was separated into four portions; one portion was used for the analysis of the fresh insect, the second portion was sun-dried, the third portion was oven dried, and the fourth portion was dried (sun-drying and oven-drying) and then defatted using a press machine. Sun-drying was attained by spreading the insects on an aluminium foil and exposing them to the sun for three days, 8 hours per day (exposure temperatures about 25°C). Oven drying was done at 60°C for 48 hours. Defatting was done by placing the dried samples in a bag in the press machine, where oil was expelled from the insects mechanically by continuous pressure on the machine. All insect samples were grinded well using a mechanical blender, transferred into airtight containers with proper labelling, and stored in a freezer at −21°C.

2.2. Proximate analysis

Moisture, ash, protein, fat and fibre analysis followed the AOAC methods (2006). Moisture, fibre, fat and ash were reported in %, while protein was reported in g/100 g.

2.3. Mineral analysis of the samples

Mineral analysis (Ca, K, Zn, Fe, and Na) was performed by Atomic absorption spectroscopy (AOAC, 2006). A sample of 0.3 g was digested using a nitric acid-hydrogen peroxide mixture (5 ml 50% conc. HNO_3 and 10 ml 30% conc. H_2O_2). Filtration was done, and the filtrate was used for AAS analysis at specific wavelength for each mineral. Standards were prepared using the commercial standard solutions of the minerals. The results were expressed in mg/100 g.

2.4. Anti-nutrient analysis of the samples

The method described by Olatoye and Arueya (2019) was used in tannin content determination. Extraction was done using 50% methanol. One millilitre of the sample extract was placed in a 50 ml volumetric flask where 2.5 cm^3 Folin-Denis reagent, 20 cm^3 distilled water and 10 cm^3 of sodium carbonate (17%) was added, mixed thoroughly and then left to stand for about 30 minutes awaiting a blue-green colour to appear. Standard stock solutions were prepared using tannic acid. Working standards in the 20–100 ppm range were made by appropriately diluting the stock solution. A UV-VIS spectrophotometer was used to obtain the absorbances of the sample and standards.

For oxalate content determination, extraction was done using 3 M H_2SO_4. The extract was titrated against hot (82–88°C) $KMnO_4$ (0.1N) to a point where a light pink colour was obtained. The calculation of oxalate content was as follows: 1 ml of $KMnO_4$ (0.1N) = 0.006303 g oxalate.

Phytate content determination was done according to the method described by Agbaire (2011). Extraction was done using 3% Trichloroacetic acid. 30 ml of 2 M NaOH was added and the precipitate was converted to insoluble ferric hydroxide and soluble sodium phytate. Hot 3.2N HNO_3 was added to dissolve the precipitate and the absorbance read at 480 nm without delay. The standard solutions in the 20–100 ppm range were made using Iron (III) nitrate. UV-VIS spectrophotometer was used to obtain the absorbances of the sample and standards. Anti-nutrient results were expressed in mg/100 g.

2.5. Statistical analysis

The raw data obtained were tabulated on a Microsoft Excel spreadsheet and expressed as the mean ± standard deviation. One-way ANOVA was performed to compare the means of the different concentrations, followed by Tukey's post hoc test to compare concentrations. All the significance tests were executed at a 95% confidence level. The analyzed data was presented in tables.

3. Results and Discussion

3.1. Proximate composition

The composition of moisture, ash and crude protein in fresh and processed grasshoppers and termites is given in Table 1.

Processing significantly reduced the moisture contents of the fresh termites and grasshoppers to less than 10%. The moisture content for the fresh grasshoppers and termites was 55.01% and 40%, respectively. Still, oven-dried grasshoppers had moisture content ranging from 3.42% to 4.00%, consistent with the reported values on oven-dried grasshoppers by Rumpold and Schlüter (2013), which gave a mean moisture content of 4.42%. For the dried termites, moisture content ranged from 1.5% to 3.7%, comparable to the 1.7% reported by Kinyuru et al. (2015) for the sun-dried termite consumed in western Kenya.

Table 1. Proximate composition of processed termites and grasshoppers.

Insect	% Moisture Content	% Ash	% Fibre	% Fat	Protein (g/100g)
Grasshoppers					
Fresh grasshoppers	55.01 ± 2.35^{a}	2.8 ± 0.38^{a}	3.9 ± 0.01^{a}	24.9 ± 0.10^{a}	16.24 ± 0.88a
Sun-dried grasshoppers	7.05 ± 0.05^{b}	3.8 ± 0.13^{b}	4.20 ± 0.20^{ab}	27.5 ± 0.10^{b}	42.31 ± 0.20b
Oven-dried grasshoppers	4.00 ± 0.51^{c}	4.01 ± 0.24^{bc}	4.20 ± 0.11^{ab}	26.7 ± 0.10^{c}	45.53 ± 0.24c
Defatted sun-dried grasshoppers	6.68 ± 0.64^{b}	3.05 ± 0.09^{ad}	3.64 ± 0.14^{c}	2.2 ± 0.1^{d}	44.11 ± 2.26bcd
Defatted oven-dried grasshoppers	3.42 ± 0.88^{c}	2.29 ± 0.09^{ae}	4.29 ± 0.22^{ab}	2.3 ± 0.1^{d}	46.02 ± 0.65cd
Termites					
Fresh termites	40.00 ± 0.12^{a}	2.23 ± 0.32^{a}	5.90 ± 0.10^{a}	44.3 ± 0.10^{a}	19.20 ± 0.24a
Sun-dried termites	3.70 ± 0.03^{b}	2.96 ± 0.18^{b}	6.37 ± 0.01^{b}	44.2 ± 0.10^{a}	33.00 ± 0.07b
Oven-dried termites	1.50 ± 0.05^{c}	4.31 ± 0.40^{c}	6.71 ± 0.10^{c}	46.4 ± 0.10^{b}	35.20 ± 0.40c
Defatted sun-dried termites	4.02 ± 0.18^{d}	1.35 ± 0.05^{d}	6.80 ± 0.01^{c}	1.90 ± 0.2^{c}	37.22 ± 0.10d
Defatted oven-dried termites	3.28 ± 0.14^{e}	0.86 ± 0.08^{d}	5.83 ± 0.12^{a}	2.00 ± 0.2^{c}	39.01 ± 0.10d

NB: Values represent mean ± S.D. ($n = 3$). Values with superscripts of different letters in the same column per each insect are significantly different.

The differences in moisture content may be attributed to the use of different drying conditions, species differences, age and habitat of the insects (Ekop et al., 2010). Another factor affecting moisture levels is the environment in which the food substance is dried. For instance, drying on bare ground makes the entire drying process cumbersome owing to water accumulation around the food substance (Saka, 2015). The moisture content of the insects was low compared to chicken and beef products (Usman & Yusuf, 2021). This means that insects have a higher dry matter content, hence a denser source of nutrients. Additionally, it reduces the availability of insects for degradative reactions initiated by spoilage microorganisms (Grabowski, 2020).

Processing methods significantly increased the ash content from 2.8% and 2.23% to 4.01% and 4.31% for grasshoppers and termites, respectively (Table 1). The ash values obtained from the grasshoppers were consistent with those in the range of 2.1% to 6.8% of other edible insects (Rumpold and Schlüter (2013); (Ssepuuya et al., 2019). Oven-dried termites reported ash content ranging from 3.91% to 4.71%, which was comparable to 3.7% indicated for dried winged termites of *Nasutitermes spp*. reported by Kinyuru (2014) and 5.5 ± 3.5% reported by Raubenheimer and Rothman (2013). Oven-dried insects had higher ash content than sun-dried insects implying the highest mineral content.

Processing did not affect the crude fibre content in the edible insects. The crude fibre of fresh grasshopper (*Ruspolia differens*) was 3.9% which was consistent with values reported in a different study for same insect species (Ssepuuya et al., 2019). Processing led to a 0.2–90% decrease in crude fat with sun-dried edible insects having the smallest reduction. Fresh *Ruspolia differens* crude fat was close to 17.8% reported for mealworms (Sayed et al., 2019) but different from the levels reported elsewhere for the same insect species (Ssepuuya et al., 2019). These variations could be linked to variations in feed substrate, age, sex, processing method and collection sites (Ssepuuya et al., 2019).

The protein content of the fresh and processed insect samples was significantly different (Table 1). The protein content for the grasshoppers and termites ranged from 16.24 ± 0.88 g/100 g to 46.01 ± 0.65 g/100 g, 19.2 ± 0.24 g/100 g to 39.01 ± 0.10 g/100 g and increased significantly on processing. The value for the fresh grasshoppers was comparable to the values within the range of 12.1 g/100 g to 26.8 g/100 g reported for different fresh grasshopper species (Ssepuuya et al., 2019), while for the fresh termites (19.2 g/100 g) was higher than 14.2 g/100 g reported by Shockley and Dossey (2014) but comparable to (20.4–22.1 g/100 g) reported for different species of the insect (Banjo et al., 2006).

The wide variability of the protein composition in this study has been linked to insect species and feeding substrate, collection sites (Scala et al., 2020) and swarming seasons, age, sex and the processing method (Ssepuuya et al., 2019). This could explain the differences between the current study findings and those reported earlier. Processes such as sun-drying, oven-drying and defatting may lead to a considerable loss of composition, for instance, moisture and fat, consequently making the other nutritional elements to concentrate (Madibela et al., 2007). Generally, the protein levels for the insects studied were higher than those of many conventional foods of both plant and animal sources, as reported by Belluco et al. (2013). Hence these edible insects can thus be considered a good protein source viable for protein supplementation in deficient diets.

3.2. Mineral content

Calcium, iron and zinc content in fresh and processed insects is given in Table 2.

There was a significant difference in calcium content between the fresh and processed samples, with processing reducing calcium by 10%–56% (Table 2). However, defatting of dried samples did not significantly lower the content, as Madibela et al. (2007) reported. The calcium content for the termites ranged from 128.50 ± 3.15 mg/100 g to 56.17 ± 0.71 mg/100 g, which was lower than 132 mg/100 g reported by Rumpold and Schlüter (2013) but was within levels of 58.72 mg/100 g as reported by Kinyuru et al. (2013). Banjo et al. (2006) reported 42.16 mg/100 g calcium content for oven-dried grasshoppers, comparable to 43.19 ± 0.56 mg/100 g detected in the oven-dried samples. Calcium content in most edible insects has been reported to be low compared to the Recommended Daily Allowance -RDA (Ghosh et al., 2017; Weru et al., 2021) for all age groups (Kinyuru et al., 2010). This is because insects lack a mineralized skeleton. Nevertheless, if insects are consumed as a supplement to the main foods, they will still offer significant additional calcium sources.

There was no significant difference in the levels of sodium, potassium iron and zinc in fresh and after processing insect samples (Table 2). The zinc content for the sun-dried termites was 22.93 ± 6.40 mg/100 g and compared well with the value of 21.79 mg/100 g for sun-dried termites reported by Anyuor et al. (2021), while sun-dried grasshoppers had zinc content of 22.06 ± 2.02 mg/100 g which closely resembled values within the range 18.64–21.79 mg/100 g

Table 2. Mineral content (mg/100 g) of fresh and processed insect.

Insect	Sodium	Potassium	Calcium	Iron	Zinc
Termites					
Fresh termites	125.01 ± 0.00^{a}	260.10 ± 2.10a	128.50 ± 3.15^{a}	8.82 ± 0.80^{a}	34.58 ± 8.35^{a}
Sun-dried termites	123.61 ± 2.20^{a}	259.60 ± 1.10^{a}	66.96 ± 2.35^{b}	8.67 ± 0.63^{a}	22.93 ± 6.40^{a}
Oven-dried termites	126.01 ± 1.10^{a}	259.40 ± 0.10^{a}	56.17 ± 0.71^{c}	7.80 ± 1.59^{a}	27.60 ± 9.36^{a}
Defatted sun-dried termites	125.62 ± 0.03^{a}	244.61 ± 2.52^{b}	64.17 ± 1.14^{b}	7.99 ± 1.92^{a}	32.50 ± 4.41^{a}
Defatted oven-dried termites	129.5 ± 0.10^{a}	260.52 ± 0.30^{a}	56.28 ± 0.71^{c}	8.71 ± 0.89^{a}	24.98 ± 1.24^{a}
Grasshoppers					
Fresh grasshoppers	300.30 ± 0.01^{a}	341.30 ± 0.03^{a}	61.05 ± 2.97^{a}	8.60 ± 3.36^{a}	19.14 ± 0.95^{a}
Sun-dried grasshoppers	301.60 ± 0.04^{c}	341.91 ± 0.01^{a}	54.42 ± 0.36^{b}	6.33 ± 2.77^{a}	22.06 ± 2.02^{a}
Oven-dried grasshoppers	300.50 ± 0.03^{ab}	342.33 ± 0.02^{a}	43.19 ± 0.56^{c}	4.36 ± 0.76^{a}	14.76 ± 2.13^{a}
Defatted oven-dried grasshoppers	290.42 ± 0.03^{d}	341.22 ± 0.66^{a}	43.51 ± 2.15^{c}	6.43 ± 1.40^{a}	22.23 ± 6.58^{a}
Defatted sun-dried grasshoppers	300.21 ± 0.04^{a}	283.01 ± 022^{b}	54.08 ± 1.34^{b}	8.89 ± 1.65^{a}	27.43 ± 1.08^{a}

NB: Values represent mean ± S.D. (n=3). Values with superscripts of different letters in the same column per each insect are significantly different.

reported by Paul et al. (2014) on sun-dried grasshoppers. Potassium was the most abundant mineral followed by sodium, which was consistent to a report by Sprangers et al. (2017) for similar insect species.

Iron content for the termites ranged between 7.80 ± 1.59 mg/100 g and 8.82 ± 0.81 mg/100 g and was lower than 24.6 mg/100 g reported by Kinyuru (2014) on oven-dried termites. The finding that processing does not affect iron and zinc is of nutritional significance. It suggests that processed edible insects can be incorporated into food formulations such as porridge powder without reducing their mineral value (Madibela et al., 2007). The micro-nutrient content of the insects was higher than that of commonly consumed beef, pork and chicken (Magara et al., 2021). Hence these insects can be considered helpful in mitigating the threat of micronutrient deficiencies in infants, young children and women of childbearing age.

3.3. Anti-nutrients levels

Table 3 gives the levels of anti-nutrients in fresh and processed termites and grasshoppers.

There was a significant difference between the fresh and processed insects' tannin, phytate and oxalate content (Table 3). The anti-nutrient content was significantly reduced on processing by 2%–70%. The values for the tannins in oven-dried termites in the current study were 0.72 ± 0.01 mg/100 g, which compared well with values for oven-dried *Macrotermes faciger* and *Macrotermes nigeriensis* termite species that had tannin content ranging from 0.47 mg/100 g to 170 mg/100 g (Kunatsa et al., 2020; Oibiokpa et al., 2017). Studies on edible insects in sub-Saharan Africa established that the tannin content of various oven-dried grasshopper species ranged between 0.72–4.35 mg/100 g (Madibela et al., 2007; Mutungi et al., 2019). However, these were higher than the current study findings, which posted 0.57 ± 0.01 mg/100 g. These variations could depend on the insect species and their feeding patterns (Hassan et al., 2009). The presence of tannins inhibits the action of trypsin and chymotrypsin hence reducing the protein digestibility. They form insoluble complexes, thereby interfering with the bioavailability of proteins. The decrease in tannins after processing treatments could result from destroying protein-tannin complexes at elevated temperatures (Essack et al., 2017). Since the levels of tannins were below the permissible limits of 150–200 mg/100 g (Master et al., 2017) in all the insects analyzed, they may not affect the digestibility of proteins obtained from these insects.

Table 3. Anti-nutrient content (mg/100 g) of processed insect.

Sample	Tannins	Phytates	Oxalates
Termites			
Fresh termites	1.69 ± 0.01[a]	0.67 ± 0.01[a]	7.88 ± 0.02[a]
Sun-dried termites	0.95 ± 0.01[b]	0.66 ± 0.01[b]	4.91 ± 0.00[b]
Oven-dried termites	0.72 ± 0.01[c]	0.24 ± 0.01[c]	4.27 ± 0.02[c]
Defatted sun-dried termites	0.77 ± 0.01[c]	0.29 ± 0.01[c]	6.57 ± 0.01[ad]
Defatted oven-dried termites	0.76 ± 0.01[d]	0.31 ± 0.01[d]	5.97 ± 0.01[bd]
Grasshoppers			
Fresh grasshoppers	0.93 ± 0.01[a]	0.41 ± 0.01[a]	14.03 ± 0.03[a]
Sun-dried grasshoppers	0.60 ± 0.01[b]	0.35 ± 0.01[b]	5.65 ± 0.08[bc]
Oven-dried grasshoppers	0.57 ± 0.01[c]	0.18 ± 0.01[c]	3.83 ± 0.05[b]
Defatted oven-dried grasshoppers	0.83 ± 0.01[ac]	0.39 ± 0.01[d]	6.43 ± 0.06[c]
Defatted sun-dried grasshoppers	0.86 ± 0.1[ad]	0.37 ± 0.003[d]	6.75 ± 0.05[c]

NB: Values represent mean ± S.D. ($n = 3$). Values with superscripts of different letters in the same column per each insect are significantly different.

The oxalate content for the oven-dried grasshoppers was 3.83 ± 0.05 mg/100 g, which was lower than the 8.28 mg/100 g reported for oven-dried *Zonocerus variegatus* grasshopper species (Omotoso & Adesola, 2018). Oven-dried termites posted oxalates values of 4.27 ± 0.02 mg/100 g, higher than 0.270 mg/100 g reported for oven-dried termites in Nigeria (Idowu et al., 2019). This variation might be due to their feeding patterns, geographical locations and variation in species (Bikila et al., 2020). The decrease in oxalates with processing aligns with earlier studies, which showed that oxalate is easily destroyed during food processing using heat treatment (Madibela et al., 2007). This is because oxalates are naturally thermally unstable. Findings by Kunyanga et al. (2011) further revealed that elevated temperatures decreased vegetable oxalate levels. The permissible limit for oxalates is 250–500 mg/100 g (Master et al., 2017). The findings from this study were however far below this limit.

Phytate content was in the range of 0.18 ± 0.01 mg/100 g to 0.41 ± 0.01 mg/100 g and 0.24 ± 0.01 mg/100 g to 0.67 ± 0.01 mg/100 g for the grasshoppers and termites respectively. Omotoso (2015) reported that the phytate content of oven-dried grasshoppers ranged from 2.81 mg/100 g to 26.49 mg/100 g, while for the termites, it ranged from 0.09 mg/100 g to 15.21 mg/100 g. These values are in line with the current study findings. Further these values are below the acceptable limits for phytate which is 250–500 mg/100 g (Master et al., 2017). Generally, due to the low phytate content in these insects, their use as food may consequently not affect the bioavailability of minerals obtained from these insects.

4. Conclusion

Moisture content reduced significantly to less than 10% on processing, while ash content increased significantly by more than 50% on processing. Processing did not affect the crude fibre content but it led to a 0.2–90% decrease in crude fat. The processing methods of oven-drying, sun-drying and defatting significantly increased the protein content in all insects in the order: defatting > oven-drying > sun-drying, but did not significantly influence mineral levels; however calcium content was reduced considerably. Anti-nutrient levels decreased significantly on processing by 2%–70% with oxalates and phytates having the highest decrease. These results show that oven-drying and defatting methods retained higher nutrient composition in the edible insects.

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Disclosure statement

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Author contributions

Anne Gachihi did experimental work, analysis of data and manuscript preparation. Prof. Hudson Nyambaka, Prof. Judith Kimiywe and Dr Chrysantus Tanga supervised the manuscript's experimental work and revision and approval.

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