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A comparison of the bioaccessible calcium supplies of various plant-based products relative to bovine milk

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A B S T R A C T

Calcium deficiency is widespread globally, especially in diets with minimal consumption of dairy. It is therefore important to identify plant-based sources of calcium that can make a meaningful contribution to calcium intakes for populations following diets with a minimum supply of dairy products. The best sources of calcium have a high calcium content and bioavailability. Therefore, we evaluated the gross and bioaccessible calcium supplies of 25 plant-based products from 5 food groups considered to be good and important sources of calcium. Bioaccessible calcium was examined using the INFOGEST static digestion model in which isotopically labelled ^{43}Ca was used as a tracer of reagent calcium to improve accuracy of bioaccessibility measurements. The gross calcium content varied widely amongst all the food products, ranging between 7.48 and 959 mg/100 g fresh weight (fw), with approximately 50 % of the products being equivalent to or surpassing the calcium content of skimmed milk. Bioaccessibility of calcium was equally variable, ranging from about 0.1 – 50 %. The lowest bioaccessibility (<10 %) was found in spinach, plant-based beverages, tofu, dried figs and tahini and was attributed to: 1. the high content of oxalate and phytate in some of the products, and 2. the low solubility of tricalcium phosphate which was used for fortification in the plant-based beverages. The remaining products generally had a high bioaccessibility that was similar to, or higher than that of skimmed milk (~30 %). When both bioaccessibility and recommended serving portions were considered, only 3 products were identified as good sources of calcium, requiring 0.2 – 1.4 servings to equal the bioaccessible supply from skimmed milk. The top three sources of plant-based calcium identified were kale, finger millet and fortified white bread in that order, with kale providing 5 times more bioaccessible calcium than 1 serving of skimmed milk. Moderate sources of calcium where 1.5 – 3 servings was equivalent to 1 serving of skimmed milk included wholemeal bread, some bean varieties (black chickpeas, chickpeas, kidney beans, peas), broccoli, cabbage and almond drink. The rest of the products were either of low calcium content, poor bioaccessibility, and/or not consumed in sufficient quantities to make a significant contribution to daily requirements. White bread was a good source of calcium as it was fortified with calcium carbonate and this suggests that mandatory widescale fortification of staple cereals with this form of calcium should be considered a viable approach to augment dietary calcium intakes in vulnerable populations. Low bioaccessibility of fortified calcium in plant-based beverages, often marketed as good sources of calcium, suggests the need for regulation and for further in vivo studies to validate bioavailability of calcium in these products.

1. Introduction

Calcium (Ca) is an essential element required to support several biological functions in humans and is especially recognised for its importance in bone health (Cormick & Belizán, 2019; Li et al., 2018). Calcium needs to be obtained from the diet with several good sources available to support a variety of lifestyles (Weaver, Proulx, & Heaney, 1999). However, in both high and low-income countries (HICs and LICs), sub-optimal intake of dietary Ca is widespread, increasing vulnerability of the population to chronic illnesses such as osteoporosis in later life (Miller, Jarvis, & McBean, 2001; Shlisky et al., 2022). Although Ca deficiency risk is substantial in HICs such as the US, of the 3.5 billion people at risk of deficiency globally, 90 % live in Africa and Asia (Kumssa et al., 2015). In Sub-Saharan Africa (SSA), about 54 % of the population have been estimated to be Ca deficient based on food supply data (Joy et al., 2014). Animal source foods, especially dairy products and fish are considered the best, and most common sources of Ca globally, providing about 72 % of dietary Ca in the food supply of HICs such as the US and more than 50 % in SSA (Joy et al., 2014; Miller et al., 2001).

For sustainability and health reasons, it is currently recommended to increase consumption of plant-based foods and reduce animal source products particularly in HICs where livestock production and consumption is unsustainable (Willett et al., 2019). However, a reduction in the consumption of dairy products in particular may increase the risk of Ca deficiency in all sections of the population as meeting Ca dietary recommendations is difficult in the absence of dairy foods (Palacios et al., 2021). The risk could potentially be greater for adolescents and

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young adults who are more likely to adopt vegetarian eating patterns than older adults (Vergeer et al., 2020) and are at an important stage in their development where bone mass is growing rapidly and peak bone mass expected to be achieved around the ages of 16 – 26 (Li et al., 2018; Miller et al., 2001). Whilst animal sources such as dairy and canned tinned fish with soft bones are the best sources of dietary calcium, plant-based foods i.e., dark green vegetables (kale, cabbage, broccoli, mustard greens) and foods processed with the aid of Ca e.g. Ca-set tofu and lime processed maize products, are also good sources (Knight et al., 2023; Weaver et al., 1999). In addition, the current diet transition has led to the proliferation of a plethora of Ca-fortified foods and beverages to supplement Ca in these products and provide adequate Ca for people avoiding dairy products.

For a food to be considered a good source of Ca, it must have a high Ca concentration and the Ca must be highly bioavailable. Dairy products have traditionally been considered excellent sources of Ca due to both a high Ca density and bioavailability. For example, a glass of 240 mL milk is estimated to contain 300 mg Ca, providing about 96 mg absorbable Ca and a bioavailability (sometimes referred to as fractional absorption) of 30 %, which is considered to be high (Weaver et al., 1999). In the case of plant-based products, most cannot match both the high Ca density and bioavailability of milk. Whilst some dark green vegetables match or even surpass the Ca density and bioavailability of milk, these are normally consumed in smaller quantities such that the amount of absorbable Ca per serving achieved per day remains lower than that of a milk serving. Dietary recommendations from most public health bodies such as the UK National Health Service (NHS) are based on the gross Ca density of foods and loosely consider bioavailability as a critical factor in determining a good Ca source. In fact, current recommendations do not acknowledge the huge disparities of Ca bioavailability in plant-based foods and are based on assumptions that have not been widely validated.

Ca bioavailability in foods is modulated by several factors. The most well-known being oxalates found in some vegetables like spinach and rhubarb, and phytates in cereals, legumes, and nuts. Oxalates and phytates bind Ca into insoluble unabsorbable salts in the gastrointestinal tract, such that Ca bioavailability is inversely proportional to their concentration in food (Amalraj & Pius, 2015; Shkembi & Huppertz, 2021). Equally, Ca bioavailability of many plant-based products will be expected to vary due to the disparity of phytate and oxalate concentrations in crops which may differ depending on the type of soils and environment in which they are grown, including the agronomic practices employed (Gabaza et al., 2007). In terms of Ca fortified foods, properties such as the solubility of the Ca compound used play a huge role in modulating Ca bioavailability with organic Ca compounds such as calcium lactate and malate more bioavailable than the phosphate and carbonate forms (Lorieau et al., 2018; Theobald, 2005). In addition, solid foods like cheese and bread with slow gastric transit seem to exhibit a higher Ca bioavailability than liquid and semi-liquid foods due to the controlled release of gastric solubilised Ca in the intestinal tract (Shkembi & Huppertz, 2021).

Dietary recommendations for Ca vary from country to country but broadly range between 700 – 1200 mg/day for adults (>19 years) (Shlisky et al., 2022; Theobald, 2005). Considering this high requirement, it is imperative to identify good plant-based sources of Ca that can be incorporated in healthy and sustainable diets. Such sources should inevitably be high in both Ca density and bioavailability, suggesting the need to have more Ca bioavailability data alongside gross Ca data currently available for most foods. Therefore, the objective of this study was to assess the bioaccessibility (or in vitro bioavailability) of several plant-based food products most of which are considered to be good and important sources of Ca for people following vegan eating patterns in HICs and for vulnerable populations in LICs. The findings from this study will help shape dietary recommendations and policies to improve dietary Ca intake and avoid adverse health outcomes related to Ca deficiency in population groups following eating patterns with non or minimal dairy products. The term bioaccessibility will be used to refer to studies conducted in vitro, while the term bioavailability will be used in discussion of in vivo studies and in reference to the wider subject area.

2. Materials and methods

2.1. Preparation of products

We consulted the NHS website for guidance on the best plant sources of Ca for a vegan diet. In addition, some cereals which are an important source of Ca for many low-income countries were also selected. The food products were categorised into groups as follows: 1. Cereals (maize, finger millet, white and brown bread, rice, oats), 2. Legumes (kidney beans, lentils, chickpeas, black chickpeas, garden peas, calcium-set tofu), 3. Dark green vegetables (broccoli, cabbage, kale and spinach), 4. Plant-based beverages (soy, rice, oat and almond milk), and 5. Dried fruits & other (raisins, prunes, figs, apricots, tahini).

All ingredients were procured from supermarkets in UK during the period July – August 2022 and prepared according to manufacturer's instructions or as common dishes. For example, maize and finger millet flour were prepared into thick porridges, a typical staple food consumed in several countries in Sub-Saharan Africa. Oat porridge and basmati rice were cooked according to instructions on the packet, using water instead of milk for the oat porridge. Dark green vegetables were steamed briefly. Skimmed milk powder was reconstituted with water according to manufacturer's instructions. All other ingredients were procured and analysed without further treatment. No cooking was required for the legumes as canned versions were used. Each food product was divided into two aliquots; one for analysis of moisture content and gross Ca composition and a second for in vitro digestion.

2.2. In vitro digestion

In vitro digestion was performed on the cooked products to accurately estimate digestive effects in the same structure/matrix in which the food is commonly consumed. All chemicals, digestive enzymes and bile were procured from Sigma (Dorset, UK) unless otherwise stated. The INFOGEST static digestion method was used (Brodkorb, Egger, & Alminger, 2019), with modifications in which a stable isotope of ^{43}Ca was added to the digestion fluids to improve measurement accuracy by enabling discrimination between reagent and sample Ca for each food digested. A similar approach has been used for iron and zinc bioaccessibility measurements and found to yield reliable and accurate data (Muleya et al., 2021).

Master-mixes of the digestion fluids were prepared i.e., simulated salivary fluid (SSF) including α-amylase from Bacillus sp. (specific activity 1380 U/mg), simulated gastric fluid (SGF) including pepsin from porcine gastric mucosa (specific activity 3412 U/mg), simulated intestinal fluid with pancreatin from porcine pancreas (specific activity 3.2 U/mg) and bovine bile (bile salt concentration of 1.4 mM/g). In each master-mix ^{43}Ca (86.9 $\pm$ 0.8 % enrichment level) was added at a level 10 $\times$ the concentration of ^{43}Ca naturally present in each fluid. In terms of the oral phase, the ^{43}Ca was added separately to the $CaCl_2$ as the $CaCl_2$ precipitates in the oral phase master-mix. The fluids with the ^{43}Ca added were incubated in a shaking water bath at 20 °C overnight to allow for complete isotopic equilibration of the ^{43}Ca added isotope and the reagent Ca in each master-mix or in the $CaCl_2$ for the oral phase.

Solid samples were masticated using a food processor to a consistency that was observed to mimic that achieved by chewing. For each food sample, 2.5 g was weighed in which the dry matter contents were standardised to 250 mg and MilliQ water (18.2 MΩ cm) added to reach 2.5 g. For example, for a product with 50 % moisture content, 500 mg of food product + 2 mL MilliQ water were digested. The selection of the amount of dry matter needed for digestion was based on preliminary experiments (unpublished data) indicating complete release of Ca when 250 mg skimmed milk powder was used. A blank digestion without food was run alongside the digestions to account for reagent Ca.

Briefly, the oral phase was initiated by adding the oral phase master-mix and $CaCl_2$ to achieve a dilution of 1:1 food: digestion fluid. The pH was adjusted to 7 followed by a 2 min. incubation in a shaking water bath, at 37 °C, 200 rpm. This was followed by gastric digestion in which gastric master-mix was added at a ratio of 1:1 food bolus: digestion fluid, pH 3 and incubated under same conditions for 2 hr. Finally, the intestinal digestion was performed with the addition of intestinal master-mixes (with pancreatin and bile), ratio 1:1 food chyme: digestion fluids, pH 7 and incubated at 37 °C for another 2 hr. Immediately after intestinal digestion, samples were placed on ice to stop enzymatic activity.

Separation of the bioaccessible Ca fraction was done according to Lorieau et al. (2018). The cooled digesta samples were centrifuged at 4,500 × g for 15 min. followed by separation of the supernatant and pellet. An aliquot of 500 μL of the supernatant was collected into 3 kDa Amicon ultra centrifugal filters and centrifuged at 8,000 × g for 30 min. The 3 kDa filtrate was collected and considered as the bioaccessible Ca fraction and stored at −20 °C pending analysis for Ca.

2.3. Gross and bioaccessible calcium analysis

Samples aliquoted for moisture content analysis and gross Ca compositions were first freeze dried to constant weight for 48 hr. The samples were ground using a pestle and mortar to a fine particle size before being analysed for gross Ca. An aliquot of 200 mg of finely ground sample was weighed into microwave vessels followed by the addition of 6 mL 65 % nitric acid. A microwave heating programme was followed as described previously to release Ca from the food matrix (Muleya, Young, & Bailey, 2021; Muleya et al., 2021).The solutions were then diluted to < 5 % acid before analysis using a triple quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (iCAP TQ, Thermo-Fisher Scientific, Bremen, Germany). The 3 kDa filtrates were diluted 10 × in 2 % nitric acid and analysed on the ICP-MS without further microwave heating. Isotopes monitored were ^{43}Ca (applied Ca isotope) and ^{44}Ca (native Ca) for the 3 kDa filtrates and ^{44}Ca only for gross Ca compositions. Conditions used for the ICP-MS can be found in Muleya, Young, and Bailey (2021).

2.4. Data processing and analysis

For gross Ca compositions, ^{44}Ca data was converted to a gravimetric basis (mg/kg fresh weight (fw)) based on the weights, volumes and dilution factors used for analysis as well as taking into account the moisture content of the samples. Data processing to calculate bioaccessible Ca, followed the approach comprehensively described in Muleya, Young, and Bailey (2021). Briefly, ^{43}Ca and ^{44}Ca raw intensity data was corrected for drift and mass differences and subsequently converted to concentration data (mg/L) using standard calibrations. The concentration of ^{44}Ca in each sample represents the total native Ca derived from the sample and reagents whereas the concentration of ^{43}Ca represents both the applied isotope and ^{43}Ca naturally occurring at an abundance level of 0.00135. Therefore, to calculate only the applied ^{43}Ca, the native ^{43}Ca was calculated from the ^{44}Ca based its isotopic abundance and was subtrated from the total ^{43}Ca concentration for each sample. The ratio of applied ^{43}Ca remaining in each sample relative to the total that was applied was then used to calculate a specific blank correction for each sample. Bioaccessible Ca concentration was converted to a gravimetric basis then expressed as a percentage relative to gross Ca in each sample. Additionally, bioaccessible Ca was also expressed per serving portion (mg) based on recommended serving portions according to the NHS and British Nutrition Foundation (BNF). One-way analysis of variance (ANOVA, $p < 0.05$) was used to compare means for gross and bioaccessible Ca compositions. Where needed, post-hoc analysis were done using Tukey's Honest Significant Difference and all analyses were performed using XLSTAT, version 2022.4.1 (378) (Addinsoft, 2023).

3. Results

The objective of this study was to examine the bioaccessible Ca compositions of 25 plant-based foods and identify food sources matching or surpassing that of dairy to support populations on diets with no or minimal dairy products. To determine bioaccessible Ca in selected foods, we used a modified INFOGEST static digestion method in which digestion fluids were isotopically labelled with a ^{43}Ca tracer thereby enabling accurate correction of reagent Ca for each sample matrix. Skimmed milk was used as the benchmark of a good Ca source, due to its high concentration of Ca, coupled with a high Ca bioavailability. Indeed, the gross composition of Ca in skimmed milk was 121 mg/100 g fw with a bioaccessibility of 30 % as expected (Table 1). These findings are in agreement with in vivo human studies demonstrating that fractional absorption of milk and most dairy products is in the region of 30 % (Fardet et al., 2019; Weaver et al., 1999), and this demonstrates the ability of our digestion method to predict the correct magnitude of response.

3.1. Cereals

Cereals are an important staple for many populations globally, contributing substantially to Ca supply in the diet especially for people living in LICs where narrow cereal-based diets dominate. Table 1 shows the gross and bioaccessible Ca compositions of some common cereals, and how they compare to skimmed milk. The Ca gross content ranged between 7.48 and 161 mg/100 g cooked product, with maize porridge, oat porridge and rice exhibiting the lowest Ca content, lower than that found in skimmed milk. Finger millet porridge had the highest Ca content, surpassing that of skimmed milk, whilst fortified white bread and whole meal bread also had high Ca content, which was also significantly higher than that of skimmed milk. Calcium bioaccessibility was generally similar to skimmed milk, except for oat porridge which had a significantly lower bioaccessibility of about 9 % (Table 1). Although not statistically significant, bioaccessibility of finger millet porridge and wholemeal bread tended to be slightly lower than that of skimmed milk whilst that of fortified white bread, maize and rice was slightly higher. Based on the gross and bioaccessible Ca compositions, amongst the cereals studied, finger millet and fortified white bread in that order, can be considered as good sources of Ca, supplying Ca levels almost comparable to a serving of milk (Table 1). Indeed, to match the bioaccessible Ca supply from milk, one will need 1.3 – 1.4 portions, in contrast to maize porridge, oat porridge and rice where more than 10 servings is required. Wholemeal bread could be considered as a moderate source of Ca, requiring 1.8 servings to equal the supply from 1 serving of skimmed milk. Based on bioaccessible Ca supplies, a serving of finger millet porridge, which was the best sources of Ca amongst the cereals, can contribute up to 8 % of the recommended Ca intakes of an adult (Fig. 1**A**), close to the 10 % contribution from skimmed milk.

3.2. Legumes

Recommendations for a sustainable and healthy diet often includes a generous amount of a diverse range of pulses and legumes due to their high protein content (Willett et al., 2019). Ca supply of these food sources is therefore critical to achieving healthy diets. The gross Ca content of selected legumes ranged from 34.7 to 322 mg/100 g cooked product, with lowest concentrations found in peas, lentils and chickpeas (Table 1). Kidney beans and black chickpeas had high Ca content in the same range as skimmed milk, while tofu had the highest Ca content. Bioaccessibility of Ca was lowest for tofu (4 %) and highest for peas (47 %). Bioaccessibility of lentils, chickpea, black chickpea and kidney beans were in the same range as skimmed milk (18 – 26 %). Although most of the legumes matched skimmed milk in terms of bioaccessibility, their lower gross Ca composition means that they are limited Ca sources with kidney beans and black chickpeas considered as the better sources.

Table 1
Gross and bioaccessible calcium compositions of 25 plant-based products compared with bovine skimmed milk.

Food Product	Ca mg/100 g fw	Bioaccessibility %	Bioaccessible Ca per serving (mg)[1]	Servings to equivalent to milk
Cereals				
Millet porridge[2,3]	161 ± 0.60^{f}	21.8 ± 5.46^{ab}	56.3 ± 14.1^{bc}	1.3
Maize porridge[4]	7.48 ± 0.16^{a}	39.0 ± 1.88^{cd}	4.67 ± 0.22^{a}	16
Oat porridge[3]	16.2 ± 0.34^{b}	9.32 ± 1.02^{a}	2.42 ± 0.26^{a}	30
Basmati rice[4]	13.8 ± 0.43^{b}	34.5 ± 4.32^{bcd}	7.16 ± 0.90^{a}	10
Fortified white bread[4,5]	132 ± 2.42^{d}	41.7 ± 4.63^{d}	51.8 ± 5.74^{bc}	1.4
Whole meal bread[3]	138 ± 1.26^{e}	23.2 ± 7.38^{abc}	30.1 ± 9.57^{ab}	2.4
Skimmed milk	121 ± 3.73^{c}	29.9 ± 9.52^{bcd}	72.4 ± 23.0^{cd}	1
p value	*<0.001****	*<0.001****	*<0.001****	
Legumes				
Black chickpeas	108 ± 3.16^{c}	24.2 ± 3.18^{b}	39.2 ± 5.14^{a}	1.8
Chickpeas	68.4 ± 1.75^{b}	26.9 ± 5.35^{b}	27.7 ± 5.49^{a}	2.6
Kidney beans[6]	140 ± 2.88^{d}	18.7 ± 0.49^{ab}	39.3 ± 1.02^{a}	1.8
Lentils	46.9 ± 1.52^{ab}	22.6 ± 1.894^{b}	15.9 ± 1.33^{a}	4.6
Peas	34.7 ± 2.49^{a}	46.5 ± 2.42^{c}	24.2 ± 1.26^{a}	3.0
Tofu	322 ± 19.8^{e}	4.09 ± 0.59^{a}	13.2 ± 1.91^{a}	5.5
Skimmed milk	121 ± 3.73^{cd}	29.9 ± 9.52^{b}	72.4 ± 23.0^{b}	1
p value	*<0.001****	*<0.001****	*<0.001****	
Vegetables				
Broccoli	52.8 ± 0.7^{a}	59.9 ± 3.70^{c}	25.3 ± 1.56^{ab}	2.9
Cabbage	68.4 ± 1.92^{a}	47.3 ± 1.97^{bc}	25.9 ± 1.08^{ab}	2.8
Kale	959 ± 121^{c}	42.7 ± 2.37^{bc}	328 ± 18.2^{c}	0.2
Spinach	201 ± 21.3^{b}	0.13 ± 0.02^{a}	0.206 ± 0.04^{a}	352
Skimmed milk	121 ± 3.73^{ab}	29.9 ± 9.52^{b}	72.4 ± 23.0^{b}	1
p value	*<0.001****	*<0.001****	*<0.001****	
Plant-based beverages				
Almond drink[7]	379 ± 7.42^{c}	3.59 ± 0.35^{a}	27.2 ± 2.65^{a}	2.7
Oat drink[7]	154 ± 47.6^{b}	3.22 ± 0.39^{a}	9.89 ± 1.19^{a}	7.3
Rice drink[7]	120 ± 7.29^{b}	5.16 ± 0.29^{a}	12.4 ± 0.71^{a}	5.8
Soya drink	46.9 ± 1.87^{a}	3.45 ± 0.22^{a}	3.24 ± 0.20^{a}	22.3
Skimmed milk	121 ± 3.73^{b}	29.9 ± 9.52^{b}	72.4 ± 23.0^{b}	1
p value	*<0.001****	*<0.001****	*<0.001****	
Dried fruits & other				
Apricots	82.5 ± 14.3^{b}	34.7 ± 1.64^{bc}	8.59 ± 0.41^{a}	8.4
Figs	180 ± 8.43^{d}	10.7 ± 0.99^{a}	5.74 ± 0.54^{a}	12.6
Prunes	42.0 ± 3.91^{a}	43.2 ± 1.58^{bc}	5.45 ± 0.20^{a}	13.3
Raisins	61.7 ± 6.33^{b}	47.4 ± 1.55^{c}	8.78 ± 0.29^{a}	8.2
Tahini	118 ± 2.6^{c}	4.90 ± 0.23^{a}	0.29 ± 0.01^{a}	250
Skimmed milk	121 ± 3.73^{c}	29.9 ± 9.52^{b}	72.4 ± 23.0^{b}	1
p value	*<0.001****	*<0.001****	*<0.001****	

[1] Serving portions: skimmed milk – 200 mL, porridges – 160 g, rice – 150 g, bread – 94 g (2 medium slices), legumes – 150 g, vegetables – 80 g, plant-based beverages – 200 mL, dried fruits – 30 g, tahini – 5 g. Values are means ± standard deviation of 3 replicates. Means from each food group were compared with skimmed milk. Means with different superscript letters are significantly different from each other ($p < 0.05$, post-hoc Tukey's Honest Significant Different Test); [2]Finger millet; [3]Wholegrains; [4]Refined grains; [5]Fortified with calcium carbonate and also contains calcium propionate as a preservative; [6]Includes calcium chloride added as a firming agent; [7]Fortified with either di- or tri-calcium phosphate.

Up to 1.8 servings of kidney beans and black chickpeas could match the bioaccessible Ca supply of 1 serving of skimmed milk, compared to 2.6 – 5.5 servings for the remaining legumes tested. A serving of black chickpea and kidney beans can thus contribute a reasonable bioaccessible Ca supply of up to 5.6 % of daily requirements of an adult (Fig. 1**B**).

3.3. Vegetables

A healthy and balanced diet includes a good supply of vegetables, with a diverse range of vegetables supplying important micronutrients to the diet. Accordingly, low oxalate vegetables in particular the dark green vegetables e.g., brassicas such as kale or cabbage, are generally considered as good sources of Ca. The gross Ca composition of the vegetables studied was lowest for cabbage and broccoli (52.8 and 68.4 mg/ 100 g fw) (Table 1), high for spinach (201 mg/100 g fw) and highest for kale (959 mg/100 g fw). The Ca content of kale was almost 8x higher than that of skimmed milk. The Ca bioaccessibility ranged between 0.13 and 59.9 % with spinach exhibiting the lowest, while that of kale and cabbage were similar to that of skimmed milk. Bioaccessibility of broccoli was the highest, surpassing that of skimmed milk. When considering the bioaccessible Ca supplies per serving (80 g), kale was by far the best source of Ca, supplying 328 mg per serving, vs., 72.4 mg from 1 skimmed milk serving. Accordingly, one only needs 0.2 servings of kale to match the Ca supply of skimmed milk, and this amounts to almost 50 % of the recommended Ca intake of an adult (Fig. 1**C**). In terms of the Brassicas (cabbage and broccoli), up to 3 servings are needed to equal the supply from skimmed milk, which makes them moderate sources of Ca. Amongst the vegetables, the worst source of Ca was spinach, which despite a high Ca content in the same range as skimmed milk, had bioaccessible Ca which was more than 300x lower than that of skimmed milk due to its low Ca bioaccessibility (Table 1).

3.4. Plant-based beverages

Plant based beverages are increasingly becoming common as bovine milk alternatives. We analysed 4 different types of plant-based beverages (almond, rice, soy and oat drink). All except soy milk were fortified with calcium phosphates (di- and tri- calcium phosphates). Accordingly, the unfortified soy drink had the lowest Ca content (46.9 mg/100 mL) (Table 1). The oat and rice drink had similar levels of Ca to skimmed milk (120 – 154 mg/100 mL) while the almond milk had approximately 3 × more Ca than skimmed milk (379 mg/100 mL). Despite similarities in the gross Ca content, Ca bioaccessibility for all the plant based beverages were 5 – 9 × lower than that of skimmed milk (less than 5 %). Due to the low Ca bioaccessibility, none of the plant based beverages could

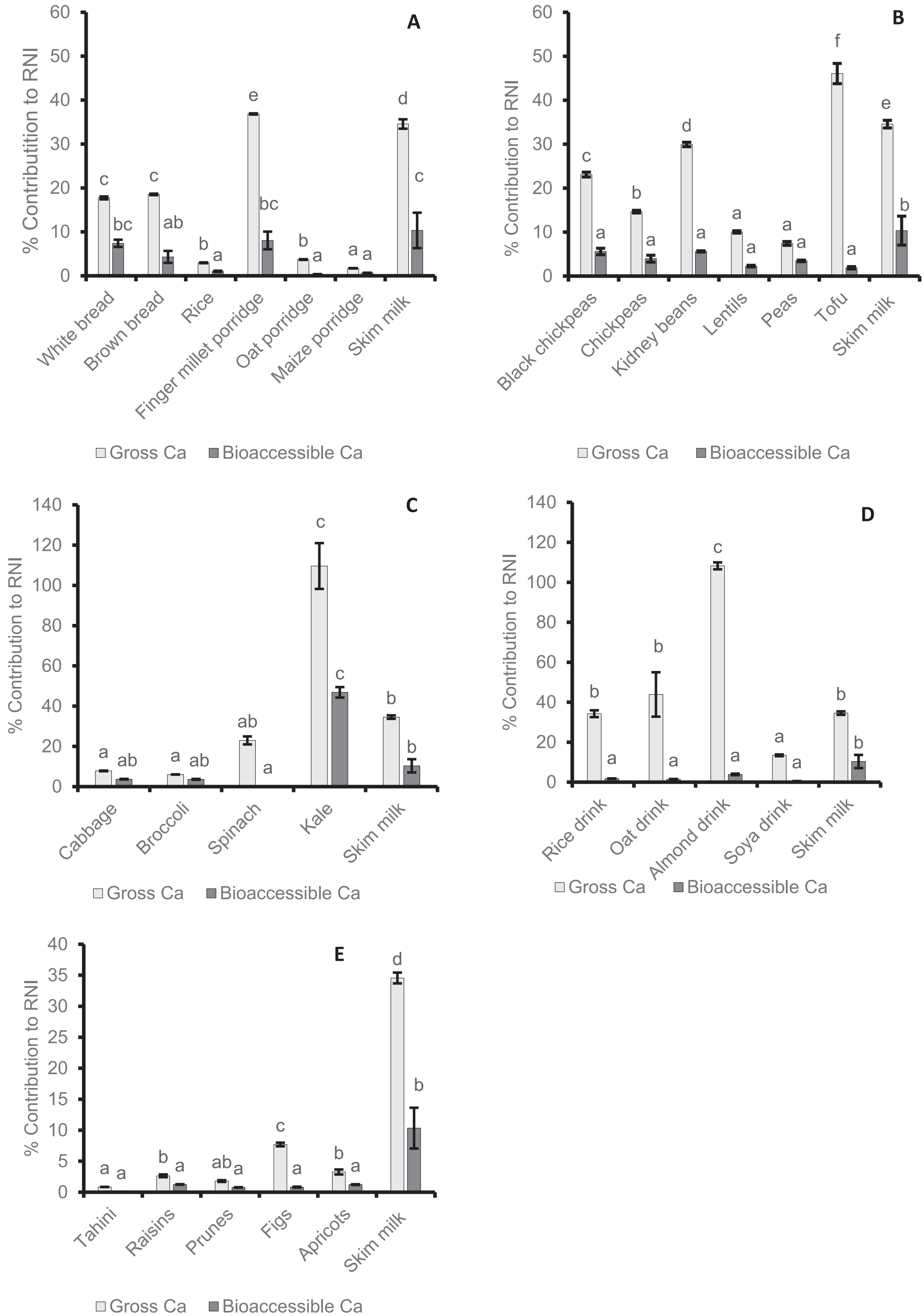

Fig. 1. A-E: Contribution of plant-based products from 5 food groups to recommended nutrient intake (RNI) for calcium based on gross and bioaccessible calcium supplies per serving.

supply bioaccessible Ca matching or surpassing that of skimmed milk. The almond drink could be considered as the better source, contributing about 3.8 % to daily adult requirements based on bioaccessible Ca supplies (Fig. 1**D**). In terms of the soy, rice and oat drink, between 5 and 22 servings are needed to match the bioaccessible supply of skimmed milk (Table 1).

3.5. *Dried fruits & others*

Within the dried fruits & others category, we analysed 4 dried fruits (raisins, prunes, figs, apricots) and tahini, a common paste derived from sesame seeds and used in a lot of culinary cuisines. These products are listed as good sources of Ca by the NHS, as part of dietary recommendation for populations following vegan diets. Calcium content ranged between 42 and 180 mg/100 g for the dried fruits and 118 mg/100 g for tahini (Table 1). Amongst the dried fruits, the dried figs contained the highest content of Ca, higher than skimmed milk, while dried prunes had the lowest Ca content. The Ca content of tahini was similar to that of skimmed milk. Although dried figs had the highest Ca content, they had the lowest Ca bioaccessibility,10.7 % (Table 1), compared to up to 47.4 % for the rest of the dried fruits. The Ca bioaccessibility for tahini was 4.9 %, compared to 30 % for skimmed milk. Although both dried figs and tahini had high Ca content in the same range with skimmed milk, due to their low bioaccessibility and lower recommended serving portion (30 g), they could not supply bioaccessible Ca equivalent to that of skimmed milk with 200 portions of tahini necessary to meet the bioaccessible supply of a single portion of milk. Likewise, dried apricots, prunes and figs could also not match the bioaccessible Ca supply of skimmed milk despite their bioaccessibility being similar to milk, with between 8 and 13 portions required to match bioaccessible supplies of skimmed milk. Consequently, a single portion of these dried fruits cannot supply more than 2 % of daily Ca requirements (Fig. 1**E**).

4. Discussion

We studied a diverse range of plant-based food products from 5 food groups in order to quantify their bioaccessible Ca supplies and evaluate the significance of their contribution to recommended Ca intakes for adults (>19 years old). Bovine skimmed milk was used as a benchmark for our comparison as milk is considered the best source of Ca, with a high Ca content and a high bioavailability (ca., 30 %). Bioaccessibility of Ca amongst all the food groups was highly variable, ranging from 0.1 to 60 % (Table 1) and seemed to depend on two factors namely, the chemical properties of the matrix and/or the chemical form of Ca used for fortification or processing in cases where this was relevant.

According to previous human studies, bioavailability of Ca in plant matrices such as cereals, legumes and vegetables could be higher or lower than that of milk depending on the phytate and oxalate content (Hambidge et al., 2005; Heaney et al., 1993; Heaney, Weaver, & Fitzsimmons, 1991; Martin et al., 2002; Weaver et al., 1991). Within the cereals food group, products prepared from whole grain cereals i.e., whole-meal bread, finger millet porridge and oats, had a lower bioaccessibility compared to those prepared from refined grains i.e., fortified white bread, rice and maize porridge (Table 1). In whole grain cereals, it has been shown that the phytate, and not the fibre per se, is the main determinant of Ca bioavailability as phytate rich fibre fractions have lower Ca bioavailability than dephytinized fibre fractions (Kennefick & Cashman, 2000; Weaver et al., 1991). This explains the higher Ca bioaccessibility of fortified white bread, rice and maize porridge as a significant amount of the phytate associated with the bran fraction was removed. On the other hand, lower Ca bioaccessibility of wholemeal bread, finger millet porridge and oat porridge were due to their higher phytate content which is associated with the bran fraction. Likewise, amongst the legumes, peas had the highest bioaccessibility of 46.5 % with the rest being in the same range as skimmed milk (18.7 – 26.9 %) except for tofu. Although phytate was not measured, peas typically have a lower phytate content than kidney beans, black chickpeas, chickpeas and lentils. This is because phytate increases with seed development, hence it will be lower in peas consumed as immature green seeds, compared to the other legumes consumed as mature dried seeds (Abebe et al., 2007; Castro-Alba et al., 2019).

The oxalate composition of the plant matrix is perhaps considered the most inhibitory to Ca bioavailability. Moreover, it is mostly concentrated in the bran fraction of grains which further explains the lower Ca bioaccessibility of the products prepared from whole grains (Siener et al., 2006). Most cereals and legumes contain oxalate although in many cases, this is not as high as in vegetables such as spinach. Oxalate content has been found to range from 3.6 to 76 mg/100 g for cereals (Amalraj & Pius, 2015; Siener et al., 2006) and 8 – 91 mg/100 g for legumes (Massey, 2007). Spinach, in contrast, can contain up to 1260 mg/100 g oxalate (Noonan & Savage, 1999; Ruan et al., 2013). Accordingly, spinach had the lowest Ca bioaccessibility, 0.1 % (Table 1), demonstrating the substantial inhibitory effect of oxalate. Amongst the dried fruits & others food group, figs and tahini had the lowest Ca bioaccessibility (10.7 % and 4.9 % respectively). Both figs and tahini contain high levels of oxalate, with sesame seeds from which tahini is derived, reported to contain up to 1315 mg/100 g oxalate (Kamchan et al., 2004). In addition, tahini also contains a high level of fat which may also modulate Ca bioaccessibility through the interaction of fatty acids with Ca to form insoluble Ca soaps during the gastro-intestinal phase of digestion (Fardet et al., 2019).

The chemical form of Ca used during food processing or fortification seems to also be an important determinant of Ca bioaccessibility. Amongst the food products studied, several were either processed with the aid of Ca e.g., calcium sulphate and calcium chloride were used as firming agents in tofu and kidney beans, respectively, or fortified with tricalcium phosphate or mixtures (di and tri- calcium) in the case of the plant-based beverages (with the exception of the soy drink). It is generally accepted that Ca fortified foods contain highly bioavailable Ca in the same range as milk (Fairweather-Tait & Teucher, 2002; Fardet et al., 2019; Shkembi & Huppertz, 2021). White bread, tortillas, dairy products and tofu enriched/processed with the main Ca compounds used in fortification (calcium sulphate, carbonate, chloride and phosphates) have been found to have a bioavailability equivalent or higher than that of milk (Shkembi & Huppertz, 2021). However, our findings indicated significant differences in bioaccessibility which are likely due to the chemical form of Ca-fortificant used. Amongst the fortified products, only white bread fortified with calcium carbonate had a bioaccessibility higher than that of skimmed milk (41.7 % vs. 29.9 %) (Table 1). In contrast, tofu and the plant-based beverages had bioaccessibility almost 6 $\times$ lower than that of skimmed milk (Table 1). Several in vitro and in vivo studies have shown a lower bioaccessibility of tricalcium phosphate compared to calcium carbonate and sulphate. For example, in a study of young women, the fractional absorption of a soy drink fortified with tricalcium phosphate was slightly lower (18.1 %) than that fortified with calcium carbonate (21.1 %) (Zhao, Martin, & Weaver, 2005). Equally, Ca bioaccessibility of tricalcium phosphate was lower than that of calcium carbonate in a rice beverage ((Silva et al., 2022) and in puffed rice extrudates and noodles (Janve & Singhal, 2018). Bioaccessibility of different chemical forms of Ca depends on their solubility under physiological conditions, especially during the gastric phase, and their molecular interactions with the food matrix in which they are embedded (Harahap et al., 2023; Shkembi & Huppertz, 2021). Tricalcium phosphate is one of the less soluble forms of Ca used for fortification and is known to sediment in soy-based beverages (Chalupa-Krebzdak, Long, & Bohrer, 2018). Bioaccessibility of Ca in the plant-based beverages could have been further worsened by the presence of phytic acid in the plant matrices. Indeed, Silva et al. (2020) showed the presence of different fractions of myo-inositol phosphates in plant-based drinks, which were particularly high in beverages based on nuts (almonds, cashews). In their study, they demonstrated that nut beverages had a bioaccessibility (measured as dialyzability) of less than 5 % which is in agreement with our findings (Table 1). Theodoropoulos et al. (2018) also showed variation in the phytic acid content of fortified soy drinks from different brands and an improvement in Ca bioaccessibility after phytase treatment, demonstrating the inhibitory effect of phytic acid on added Ca. It is not clear why the calcium sulphate in tofu was as poorly bioaccessible as the tricalcium phosphate in the plant-based beverages, although the inhibitory effect of phytates potentially present in the tofu cannot be ruled out.

The prerequisite for a food product to be a good source of Ca is a high Ca density accompanied by high bioavailability. In addition to that, the product must be consumed in sufficient quantities in order to make a meaningful contribution to the daily Ca requirements. In that regard, we calculated the bioaccessible amounts per serving of each food product analysed (Table 1). Amongst the cereals, although the low phytate maize porridge and rice were of high Ca bioaccessibility, they were still not good sources of Ca due to their inherent low Ca content. On the other hand, finger millet and fortified white bread both had a high Ca content and bioaccessibility, qualifying them as good Ca sources. The high bioaccessible Ca supply of finger millet, which is naturally gluten-free, is particularly interesting for populations who cannot consume wheat products and for populations living in arid and semi-arid regions where it is typically grown. Finger millet is grown in many parts of LICs, such as in some parts of SSA and Asia where it could make a meaningful contribution to Ca supply in the diet (Maharajan et al., 2021; Puranik et al., 2017). In fact, in these regions, serving portions will likely be larger than the ones used in this study (>150 g), suggesting an even higher contribution to recommended Ca intakes. Although fortified white bread was a good source of Ca, this is only relevant in the context of UK where fortification of white flour and not wholemeal flour is mandatory (Palacios et al., 2021). Indeed, dairy and wheat products provide about 66 % of dietary Ca intake to the UK population (Theobald, 2005). The white flour used to make the white bread in this study was fortified with calcium carbonate, where the legal requirement for fortification of flour in UK is 235 – 390 mg/100 g according to the Bread and Flour Regulations of 1998. In countries with no mandatory Ca fortification, wholemeal bread could also be a good source of Ca as it intrinsically contains a high level of Ca and has a reasonable bioavailability, providing just under 10 % of recommended Ca intakes (RNI) (Fig. 1**A**). For populations consuming a diverse range of wholemeal wheat products, this might substantially contribute to their daily Ca requirements.

Legumes, kidney beans and black chickpeas could be considered as reasonable sources of Ca based on their high Ca density and moderate Ca bioaccessibility (Table 1). However, it should be noted that a small amount of calcium chloride was added as a firming agent in the canned kidney beans although the exact amount added was not stated on the label. This could have slightly increased the Ca content of the kidney beans meaning that consumers have to look at the nutritional information on the labels as not all canned beans will necessarily contain this additional Ca. Likewise, tofu is marketed as a good source of Ca due to the application of Ca salts during processing, however not all brands of tofu are processed with the aid of Ca salts. Of particular interest in this study was the low bioaccessible Ca supplies of tofu and plant-based beverages despite high Ca contents **(**Table 1**).** Gross Ca composition of the plant-based beverages were as high as that of skimmed milk and 2 – 3 × higher for the almond milk and tofu. The low Ca bioaccessibility observed for these products is quite concerning as these products are marketed as good sources of Ca, which could augment Ca intake for populations following vegan eating patterns. One of the challenges with respect to the plant-based beverages is the lack of regulation on Ca health claims especially the lack of guidelines on the type of Ca compounds that should be used and the amounts of Ca that should be added. Indeed, recent studies indicate the high variability in the Ca content of a range of plant-based beverages (Chalupa-Krebzdak et al., 2018). In some countries, the chemical form of Ca used for fortification is not even stated on the package while other plant-based beverages are not fortified at all (Theodoropoulos et al., 2018). Regulation in these areas is therefore critical in order to protect consumers who are honestly looking to increase their dietary Ca supplies from non-dairy food sources. Nonetheless, in vivo studies may be needed to validate the bioavailability of different Ca compounds in these products.

Kale was by far the best source of Ca across all food groups, with a serving portion providing more than 50 % of the recommended Ca intakes for an adult (>19 years). Although low oxalate vegetables such as the Brassicas (broccoli and cabbage) are generally considered as good sources of Ca, due to their high Ca bioaccessibility, not all could provide substantial Ca per serving. This is because their Ca content is not as high as that of milk, which further reinforces the assertion that 'the high Ca absorbability of a particular food cannot overcome its low Ca content' (Miller et al., 2001). Furthermore, most cooked vegetables will have a high moisture content, which means the dry matter content actually consumed may be small. For example, dry matter content of cooked kale was about 31 % compared to 11 – 15 % for cabbage and broccoli, respectively. Nonetheless, broccoli and cabbage could be considered as moderate sources of Ca. It is not clear why dried fruits are considered as good sources of Ca on the NHS website as all the dried fruits analysed in this study had either a low Ca content or low Ca bioaccessibility. Moreover, the recommended serving portions of 30 g for dried fruits is too small to make a significant contribution to Ca dietary intake.

Based on the findings from this study, the narrative about non-animal sources of Ca needs to go beyond gross compositions and emphasize bioavailability as an equally key component determining dietary Ca supplies. Fig. 1**A-E** shows the stark differences between gross and bioaccessible supplies and highlights how basing supplies on gross composition alone can be highly misleading. For example, based on gross compositions alone, 10 out of 25 products analysed could potentially contribute at least 20 % of Ca to recommended intakes. However, when considering bioaccessible Ca, only 3 out of 25 products could contribute close to 10 % of recommended Ca requirements for adults. Fortifying foods with Ca without considering the bioavailability of the chemical form of Ca used and the matrix in which it is embedded may not necessarily be a silver bullet to close the dietary Ca gap in vulnerable populations. In vivo evidence is needed pertaining to the bioavailability of several Ca forms used in the fortification of plant-based beverages. On other hand, evidence of the high bioavailability of calcium carbonate in wheat flour has been reported and also confirmed in this in vitro study. Mandatory widescale fortification of white wheat flour with calcium carbonate could therefore be a useful intervention in settings where calcium deficiency is widespread and where wheat is a staple.

Overall, we have shown that only a few food sources were equivalent to milk in terms of their Ca bioaccessible supplies with kale, finger millet porridge and fortified white bread being the top three sources (Fig. 2). Amongst the 3 best sources, kale was by far the best source as it could provide up to 50 % of daily Ca requirements, for a small and low calorie serving of just 80 g. On the other hand, finger millet is an energy dense product which will need to be complemented with other sources. Identification of other dark green vegetables like kale in LICs could be important to complement supplies from finger millet or fortified cereals if available. Indeed, dark green vegetables and nixtamalized maize (processed with the addition of lime) were among the best plant-based food sources identified to meet recommended Ca intakes for populations in Uganda, Guatemala and Bangladesh (Knight et al., 2023).

5. Conclusions

The gross compositions of about 50 % of the analysed 25 plant-based products were equivalent or even higher than that of milk. However, when the bioaccessibility and recommended serving portions were considered, only 3/25 products could provide bioaccessible Ca equivalent to milk, with kale supplying 5 × more bioaccessible Ca than milk. The bioaccessible Ca supplies from plant-based beverages were surprisingly low, despite their high Ca content which raises doubt about the Ca health claims often associated with most brands. Bioaccessibility from these products was < 5 %, compared to 30 % for milk and was attributed to the low solubility of the tricalcium phosphate used for fortification and the potential presence of phytate. This shows that for fortification programmes to effectively augment dietary Ca supplies, the right food vehicle and chemical form of the calcium used must be carefully considered. White bread fortified with calcium carbonate was amongst the best source of Ca identified, indicating the potential of mandatory widescale Ca fortification in meeting recommended Ca intakes. It should be noted that we used modest recommended Ca intakes for adults (700 mg/day) to evaluate the significance of Ca supplies. However, Ca requirements are higher for adolescents (~1000 mg/day), which means even more careful considerations need to be made for individuals in this population group following diets with non or minimal

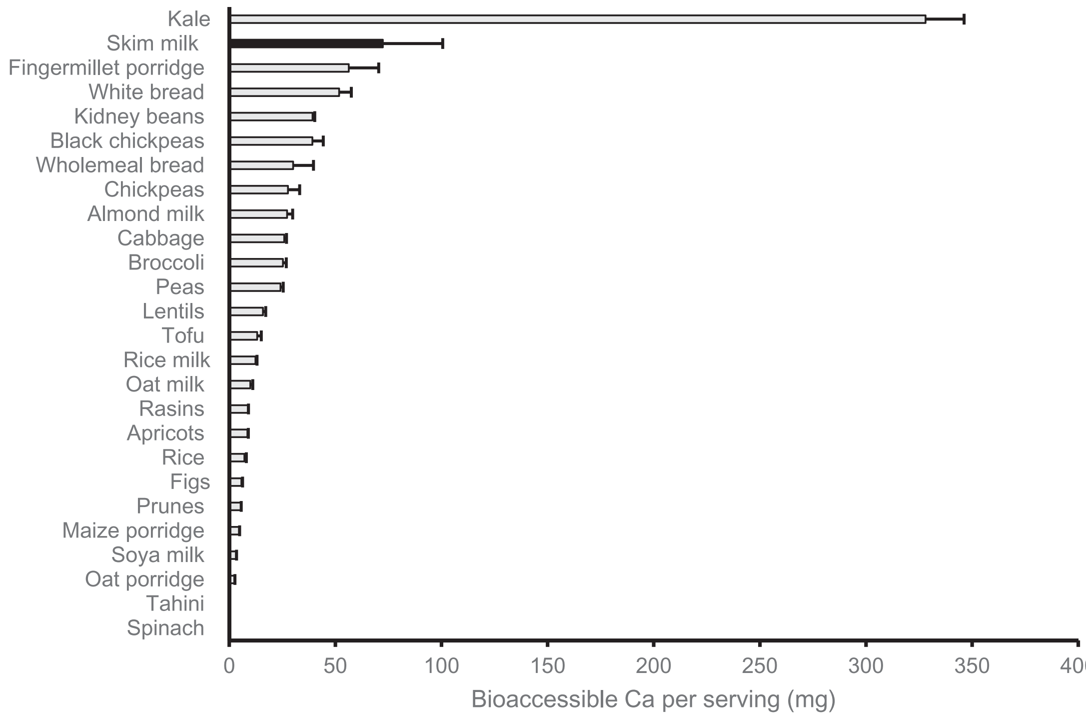

Fig. 2. Ranking of the bioaccessible calcium supplies per serving of 25 analysed plant-based products.

in dairy products.

CRediT authorship contribution statement

Molly Muleya: Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **Esther F. Bailey:** Methodology, Writing – review & editing. **Elizabeth H. Bailey:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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