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Nutritional quality of *Onobrychis viciifolia* (Scop.) seeds: A potentially novel perennial pulse crop for human use

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Abstract

Onobrychis viciifolia (hereafter sainfoin) is an autotetraploid ($2n = 4x = 28$), allogamous insect-pollinated perennial legume originating from the Caucasus that has historically been cultivated as a forage. As a perennial legume, sainfoin has the potential to improve the sustainability of agriculture and food systems in multiple ways. Sainfoin can provide continuous living cover and biological nitrogen fixation to enhance soil fertility and health. It can also provide ecosystem services as a resource for pollinators and wildlife in addition to nitrogen fixation. Building on this history of valuable uses, The Land Institute is developing sainfoin as a pulse crop for human use. With the goal of supporting human diets with a sustainable, perennial protein source and nutrient-dense crop, this innovation requires a thorough understanding of the chemical composition of sainfoin seeds to ensure safety and potential nutritional quality. Using seeds from commercial sainfoin varieties developed for forage production, grown by commercial sainfoin seed growers in the western United States, this study evaluates seed composition as part of an ongoing investigation into sainfoin's potential as a novel pulse. We found crude protein content (38.78%) comparable with soybean and lupine, fat content (6.96%) comparable with lupine and chickpea, and starch (7.1%) and dietary fiber content (48.96%) comparable with lupine. Phytic acid content was higher than pulses (1790.89 mg). Ash (3.81%), iron (64.14 ppm), and zinc contents (61.63 ppm) were in the higher end of the range for pulses. This study indicates that sainfoin could become a novel, nutrient-dense crop for human nutrition. Future studies are required to further characterize seed composition and safety and demonstrate how common legume processing techniques may influence nutritional quality.

KEYWORDS
nutrition, perennial, plant protein, pulse, sainfoin, sustainability

1 | INTRODUCTION

Onobrychis viciifolia (hereafter sainfoin) is an autotetraploid ($2n = 4x = 28$) (Frame et al., 1998), allogamous, insect-pollinated perennial legume originating from south central Asia (Carbonero et al., 2011). Sainfoin has been widely cultivated in Europe, Asia, and in certain parts of North America, demonstrating its adaptability to diverse environmental conditions (Frame et al., 1998). Sainfoin has

FIGURE 1 Sainfoin seed production in the western United States, near Twin Bridges, MT. Sainfoin fields are managed for forage production (hay), grazing, and/or seed production, depending on available soil moisture, growing season, and market demands. Additionally, fields are typically stocked with honeybees at a density of five colonies per hectare for honey production and sainfoin pollination. (a) Irrigated sainfoin field during flowering; (b) flowering sainfoin in a seed production field; (c) sainfoin legume, consisting of a single seed in each pod; (d) sainfoin seeds with seed coat covering cotyledons (i.e., whole; left) and with seed coat removed and cotyledons separated (i.e., decorticated or split; right).

been historically cultivated as a perennial forage legume with a recognized importance to the sustainability of agricultural systems (Figure 1). For instance, English covenants from the 1800s held by Cotswold Seeds Ltd document that farmers were required to grow sainfoin to maintain soil fertility, which provides evidence for the integral role of sainfoin in sustainable cropping systems as a source of biologically fixed nitrogen (Carbonero et al., 2011). With the increased availability of synthetic nitrogen, and the associated decoupling of on-farm nitrogen cycling among legumes, other row crops, and livestock, sainfoin acreage consequently declined.

Sainfoin has the potential to enhance the diversity and sustainability of agroecosystems by providing multiple revenue streams, numerous ecosystem services, and the flexibility to adapt management strategies to climate change (Carbonero et al., 2011; Nielsen, 2021). It has several distinct benefits as a forage, including reduced risk of bloat, increased protein digestibility, and reduced methane production, which are attributed to condensed tannins in the foliage (Berard et al., 2009; Carbonero et al., 2011; McMahon et al., 2000; Mora-Ortiz & Smith, 2018; Mueller-Harvey, 2006; Waghorn & McNabb, 2003; Wang et al., 2006; Wang et al., 2015). Sainfoin also has advantages as a pollinator resource, especially for bees, because it provides both pollen and nectar and has an early onset of floral resources and an extensive flowering period (Kells, 2001; USDA NRCS, 2022). Honeybees can produce an abundance of high-quality honey when managed properly on sainfoin fields (Kells, 2001; Kropacova & Haslbachova, 1970). Sainfoin, as a component of conservation plantings, contributes to soil and water conservation and wildlife habitat as part of the United States Department of Agriculture (USDA) National Resource Conservation Service (NRCS) Conservation Reserve Program (CRP) (USDA NRCS, 2022).

For these and other reasons, The Land Institute is leading efforts to develop an additional end use and revenue stream for sainfoin as a perennial pulse crop. Sainfoin can produce an abundance of seed, with reported seed yield exceeding 1000 kg/ha (Doyle et al., 1984; Hanna et al.,1970; Iwaasa et al., 2020; USDA NRCS, 2022). Like other perennial grains in development, sainfoin production is likely to have numerous agroecosystem benefits (Cox et al., 2004; Cox et al., 2006; Glover et al., 2010; Jackson, 2002); however, as a perennial nitrogen-fixing legume, sainfoin may prove to be a novel and sustainable protein source and nutrient-dense crop with human health benefits (Schlautman et al., 2018). Developing sainfoin as a perennial pulse crop will spark renewed interest and revitalize research efforts, leading to increased awareness and utilization. However, this innovation requires a thorough understanding of the chemical composition of sainfoin seeds, their nutritional quality, and potential food uses to facilitate integration into diets to sustain and improve human health.

There are few published studies detailing sainfoin seed composition. For example, Tarasenko et al. (2015) analyzed flour milled from seeds of *Onobrychis arenaria*, an intrageneric relative of *O. viciifolia*, and found that the seeds had considerable protein (28.7%) and crude fiber content (19.4%), oil content below 8%, and a pleasant taste. Baldinger et al. (2016) demonstrated that sainfoin seeds could be used in organic diets of weaned piglets and observed suitable amino acid profiles and protein content (27.9% seed and pod; 38.8% seed) within sainfoin seeds. Ditterline et al. (1977) found no significant difference in body weight gain and feed conversion ratio for rats fed soybean meal or depodded sainfoin seeds and reported that depodded seeds contain approximately 36% crude protein.

This study is part of a larger effort to determine whether sainfoin seeds are safe for human consumption. The Land Institute is preparing a dossier for submission to the Food and Drug Administration to have sainfoin seeds and seed products listed as generally recognized as safe (GRAS) ingredients in the United States. Therefore, to provide evidence for that dossier and contribute to a better understanding of sainfoin as a perennial pulse crop, this study had the following objectives:

1. Analyze the composition of *O. viciifolia* seed samples, representing commercial seed lots

2. Compare the composition to both major and minor pulses and soybean, with a focus on potential human health benefits
3. Infer relationships between seed components and between sainfoin seed samples
4. Propose future studies that will support research and development

The research conducted herein utilized seed samples from commercial sainfoin varieties developed for forage production and grown by commercial sainfoin seed growers in the western United States to support the sainfoin forage industry. To our knowledge, these are the most common, if not the only examples of sainfoin germplasm that are in production in the United States that have proven potential to consistently produce a seed and forage crop. Thus, the seeds analyzed serve as a realistic and practical example of pulse-type varieties that could be integrated into the food system. These data provide a baseline to identify breeding objectives for sainfoin as a pulse, and from which comparisons can be made to other pulses.

2 | MATERIALS AND METHODS

2.1 | Seed material

Sainfoin seed samples, representing commercial seed varieties, were sourced from sainfoin seed growers in Montana and Idaho, USA (Table 1).

2.2 | Seed analysis

Sainfoin seeds were removed from pods (i.e., depodded) before component analysis, which was performed by Great Plains Analytical Laboratories (GPAL) (Kansas City, MO, USA) unless otherwise noted. All analyses were conducted using standard, approved methods of analysis to ensure comparability with external data sets. Thousand kernel weight was quantified according to Federal Grain Inspection Service (FGIS) protocol. Information on the methods of analysis for the components by data set are provided in Table 2. Data for sugars were not available for sainfoin samples with IDs PJ-S-13-14, PJ-S-16,

TABLE 2 Components and methods of analysis by data set.

Component	FAO pulses	Sainfoin
Moisture	Gravimetric method	AACC 44–15.02
Ash	Gravimetric method	AACC 08–01.01
Protein	Kjeldahl method (total nitrogen × 6.25)	AACC 46–30.01
Crude fat	Soxhlet method (continuous extraction)	AACC 30–25.01 (ether extraction)
Carbohydrates	100 − (Ash + Moisture + Fat + Protein)	100 − (Ash + Moisture + Fat + Protein)
Total starch	-	AOAC 996.11
Dietary fiber	AOAC Prosky method	AACC 32-07.01/ AOAC 991.43
Iron	AAS, ICP, ICP-MS, and colorimetric methods	AACC 40-70.01
Zinc	AAS, ICP, ICP-MS, and colorimetric methods	AACC 40-70.01
Phytic acid	Determined by direct precipitation	HPLC RI

Note: Soybean methods could not be confidently aligned with components using information available from USDA.

TABLE 1 Seed sample ID, grower code, variety code, variety, and year ($N = 15$).

ID	Grower code	Variety code	Variety	Year
PJ-S-13-14	PJ	S	Shoshone	2013–2014
PJ-S-16	PJ	S	Shoshone	2016
CF-R-19	CF	R	Remont	2019
MG-D-19	MG	D	Remont	2019
S-D-18	S	D	Remont	2018
R-S-18	R	S	Shoshone	2018
R-D-18	R	D	Remont	2018
MG-D-18	MG	D	Remont	2018
R-R-18	R	R	Remont	2018
W-D	W	D	Remont	-
W-R	W	R	Remont	-
W-M	W	M	AAC Mountainview	-
W-Rx	W	Rx	Renumex	-
CS-E	CS	E	Eski	-
CS-S	CS	S	Shoshone	-

CF-R-19, MG-D-19, S-D-18, and MG-D-18. For the quantification of phytic acid, flour of each sample was acidified, and the analyte was isolated with solid phase extraction. The sample was then analyzed using reverse-phase high-performance liquid chromatography (HPLC) with refractive index (RI) detection.

2.3 | External data sets

Pulse crop nutritional quality data were downloaded from the Food and Agriculture Organization/International Network of Food Data Systems (FAO/INFOODS) Global Food Composition Database for Pulses (Version 1.0, uPulses1.0, 2017) (FAO, 2017). Data for raw seeds were reported on an edible portion, dry matter content basis. For comparisons, data for components in common with the sainfoin data set were selected for all species in the pulse data set, including *Cicer arietinum* (L.) (chickpea); *Lens culinaris* (Medik) (lentil); *Phaseolus vulgaris* (L.) (common bean); *Pisum sativum* (L.) (pea); *Vicia faba* (L.) (broad bean or fava bean); *Vigna radiata* (L.) R Wilczek (mung bean); *Vigna unguiculata* (L.) Walp (cowpea); *Vigna angularis* (Willd) Ohwi & H. Ohashi (Adzuki bean); *Vigna subterranea* (L.) Verdc. (Bambara groundnut); *Lablab purpureus* (L.) Sweet (Hyacinth bean); *Phaseolus lunatus* (L.) (Lima bean); *Lupinus* spp., including *L. albus*, *L. angustifolius*, and *L. luteus*; *Vigna aconitifolia* (Jacq) Marechal (Moth bean); *Vigna mungo* (L) Hepper (Mungo bean); *Cajanus cajan* (L) Huth (Pigeon pea); and *Vigna umbellata* (Thunb.) Ohwi & H Ohashi (Rice Bean).

Nutritional quality data for raw, mature seeds of *Glycine max* (L.) (soybean) were downloaded from the USDA Agricultural Research Service (ARS) FoodData Central.

Data are provided in Table S1 for the components in common among the sainfoin, pulse, and soybean data sets and in Table S2 for the components specific to the sainfoin data set.

2.4 | Statistical analyses

Thousand kernel weight and total starch (AOAC 996.11) data were analyzed for sainfoin (data not available for FAO pulses and soybean). Common components between the sainfoin, pulse, and soybean data sets were included in joint statistical analyses. Carbohydrate content was calculated by difference: $Carbohydrates = 100 - Protein - Fat - Ash - Moisture$. All seed components were adjusted to a dry matter basis using moisture content, and values are reported on an edible portion dry matter basis (EPDM). All statistical analyses were performed using the R statistical software, unless otherwise noted (R Core Team, 2022). Summary statistics were calculated using the summarized function (Wickham et al., 2022). Pearson correlation coefficients and significance values were calculated using the *rcorr* function (Harrell, 2022). Principal component (PC) analysis was performed using the *prcomp* function in base R, with data centered and scaled. A biplot of PCs was visualized using the *factoextra* package (Kassambara & Mundt, 2020).

3 | RESULTS AND DISCUSSION

3.1 | Sainfoin thousand seed weight smaller than lentil

Sainfoin thousand seed weight (TSW) ranged from 14.4 to 18.7 g, with a mean value of 17.0 g (standard deviation [sd] = 1.3 g). TSW values vary considerably among legumes. For example, Guiguitant et al. (2020) reported a range in values from 12.8 (*Trigonella foenum-graecum*) to 1178 g (*Phaseolus coccineus*). Based on their study, sainfoin appears to have a comparable average TSW to *Astragalus* sp. (17.7 ± 18.9 g) and a lower value compared with mung bean (39.9 ± 11.1 g). Sainfoin TSW appears to be smaller than the lower end of the range for lentils, with minimum values of 20.90 g reported by Dutta et al. (2022) and Verma et al. (2015) and 26 g reported by Thavarajah et al. (2008). These minimum values correspond to the extra small red market class, a typically decorticated (i.e., split) form, whereas maximum values of 42.13 g (Dutta et al., 2022), 50.8 g (Verma et al., 2015), and 62 g (Thavarajah et al., 2008) correspond to the large green lentil market class, a whole seed form. Sainfoin market classes, although not currently defined, are likely to emerge and could resemble the varied lentil market classes that exist. Lentil market classes are numerous, are broadly categorized based on whether the seed is whole or split, and are further defined based on seed size and color. Variation among sainfoin germplasm for seed size, seed weight, seed coat color, and cotyledon color would have to be present to support the development of numerous market classes.

In addition to facilitating marketing of sainfoin as a novel food crop, it is critically important to understand the extent of variation in sainfoin seed composition and morphology and relationships with agronomic traits and nutrition quality. Seed weight has been shown to be a reliable predictor of grain yield (Guiguitant et al., 2020) and is expected to have positive correlations with plant height and reproductive effort, as well as seedling biomass (Chapin et al., 1993; Fayaud et al., 2014; Renzi et al., 2022; Tamet et al., 1996; Wettberg et al., 2014). Seed size is especially important in the context of domestication, where it has likely undergone selection along with agronomic traits such as erect growth habit, both characteristics of the highest yielding species (Guiguitant et al., 2020; Smartt, 1978).

3.2 | Sainfoin protein content comparable with soybean and lupine

Proximates evaluated in this study included ash, carbohydrates, fat, and crude protein content (Table 3). Sainfoin had comparable crude protein content with soybean and lupine, and these crops had mean values well above the range of mean values for pulses. The range in sainfoin crude protein content that we found (34.95%–40.97% EPDM) is generally comparable, although slightly higher than the few instances reported in the literature. For depodded seeds, which were also analyzed in this study, crude protein values were reported by Baldinger et al. (2016) (38.8%), Woodman and Evans (1947) (36.6%),

TABLE 3 Proximates reported for pulses, sainfoin, and soybean.

Crop	N	Crude protein	Crude fat	Ash	Carbohydrates
Soybean[a]	1	39.9	21.8	5.32	32.98
Sainfoin	**15**	**38.78 ± 1.78 (34.95–40.97)**	**6.96 ± 0.47 (6.25–7.76)**	**3.81 ± 0.23 (3.44–4.19)**	**50.45 ± 1.38 (48.75–53.21)**
Lupine[b]	6	37.57 ± 4.39 (33.02–45.36)	7.09 ± 1.44 (5.55–9.73)	3.97 ± 0.74 (2.63–4.84)	51.37 ± 4.63 (44.26–56.83)
Lentil[b]	7	27.91 ± 1.26 (26.88–30.3)	1.34 ± 0.26 (1.02–1.65)	2.77 ± 0.35 (2.32–3.28)	67.97 ± 1.13 (65.5–68.76)
Broad bean[b]	2	27.81 ± 0.89 (27.18–28.44)	1.94 ± 0.58 (1.53–2.35)	3.41 ± 0.39 (3.13–3.69)	66.84 ± 0.7 (66.34–67.34)
Moth bean[b]	2	26.42 ± 0.09 (26.35–26.48)	2.24 ± 0.22 (2.08–2.39)	4.27 ± 0.15 (4.16–4.37)	67.08 ± 0.28 (66.88–67.27)
Mungo bean[b]	2	26.33 ± 0.15 (26.23–26.44)	1.51 ± 0.03 (1.49–1.53)	3.69 ± 0.03 (3.68–3.71)	68.46 ± 0.21 (68.31–68.6)
Hyacinth bean[b]	1	26.32	1.71	3.6	68.37
Pea[b]	10	24.7 ± 2.88 (20.38–28.39)	2.07 ± 0.44 (1.4–2.56)	2.75 ± 0.33 (2.01–3.17)	70.49 ± 3.5 (66.39–75.57)
Cowpea[b]	4	24.37 ± 1.65 (22.54–26.24)	2.33 ± 0.34 (2.12–2.84)	3.76 ± 0.14 (3.61–3.89)	69.54 ± 1.66 (67.78–71.64)
Common bean[b]	10	24.3 ± 0.97 (22.44–25.58)	1.85 ± 0.29 (1.46–2.35)	4.35 ± 0.56 (3.59–5.56)	69.5 ± 0.9 (68.18–70.7)
Mung bean[b]	3	24.29 ± 2.65 (22.37–27.31)	1.72 ± 0.44 (1.45–2.23)	3.57 ± 0.29 (3.38–3.9)	70.41 ± 3.37 (66.56–72.8)
Pigeon pea[b]	3	24.21 ± 0.92 (23.22–25.03)	2.1 ± 0.44 (1.68–2.56)	4.17 ± 0.25 (3.89–4.38)	69.52 ± 0.89 (68.7–70.46)
Lima bean[b]	1	23.04	1.7	4.63	70.63
Adzuki bean[b]	1	22.99	0.62	4.13	72.26
Chickpea[b]	6	22.13 ± 1.61 (19.9–23.8)	5.87 ± 0.47 (5.48–6.64)	3.03 ± 0.14 (2.79–3.2)	68.98 ± 1.91 (66.84–71.42)
Rice bean[b]	2	21.21 ± 0.05 (21.17–21.25)	0.59 ± 0 (0.59–0.59)	4.51 ± 0.03 (4.48–4.53)	73.69 ± 0.08 (73.63–73.75)
Bambara groundnut[b]	1	20.27	7.08	3.76	68.88

Note: Values reported as mean value ± standard deviation (minimum–maximum) on an edible portion dry matter (EPDM) basis; rows sorted by crude protein content.
[a]USDA ARS Food Data Central.
[b]FAO (2017).

Ditterline et al. (1977) (35.6%–36.0%), and Kling and Wöhlbier (1977) (30.3% DM). Although the USDA data set does not provide a range for soybean values, Assefa et al. (2019) assessed the variation in soybean oil and protein content for major US growing regions and reported a range in protein content from 27.3 to 45.4 g/100 g, with 90% of the data (13,574 data points total) within a 6.0 g/100 g range (i.e., 33.0 to 39.0 g/100 g) of the mean (35.7 g/100 g). Although the range in sainfoin protein content determined in this study is comparable with soybean and lupine, it may be possible that sainfoin protein content could exceed the protein content of these legumes. The sainfoin varieties analyzed in this study are a narrow representation of the sainfoin germplasm pool, which may harbor genetic diversity to facilitate breeding and selection for elevated protein content. Protein content may also be increased through best-management practices for sainfoin seed production and quality, which were not necessarily utilized to produce the seeds analyzed in this study. These practices can be developed through future agronomic studies.

3.3 | Sainfoin fat content comparable with chickpea

Sainfoin had comparable crude fat content to bambara groundnut, chickpea, and lupine, and these crops had mean values above the range for pulses. In their analysis of pulse crops, Guiguitant et al. (2020) reported a moderate positive correlation between protein and oil content ($r = 0.45$, $P < 0.01$). In soybean, a strong negative correlation is often reported between protein and oil content, as is reported by Choi et al. (2021) ($r = -0.714$, $P < 0.0001$). However, Assefa et al. (2019) found no significant correlation within a pooled soybean data set, even though a negative relationship was observed when evaluating each of the studies in the database separately. In the study by Choi et al. (2021), they reported interesting relationships between fatty acid content, especially content of the essential fatty acid linoleic acid, and soybean seed coat color and seed weight. Linoleic acid and alpha-linolenic acid are essential fatty acids (must be consumed in the diet). Given the relatively high fat content of sainfoin compared with other pulses, sainfoin could provide significant amounts of fatty acids to benefit animal and human nutrition (Jukanti et al., 2012; Madurapperumage et al., 2021). Conversely, the high fat component of the seed relative to other pulses (e.g., pea) may be a challenge to producing protein concentration or isolates, as this fraction must be removed; a lower proportion of fat is ideal for producing larger quantities of protein products (Lam et al., 2018).

3.4 | Sainfoin ash, iron, and zinc content comparable with pulses

The mean value for sainfoin ash content fell within the pulse values, which ranged from 2.75 g/100 g EPDM (pea) to 4.63 g/100 g EPDM (lima bean). Soybean ash content was slightly higher, at 5.32 g/100 g

EPDM (Table 3). Sainfoin mean iron content fell within the range of mean values for pulses and was most comparable with lupine, pigeon pea, cowpea, hyacinth bean, and rice bean, followed by pea, broad bean, and chickpea. Sainfoin mean zinc content was most comparable with hyacinth bean, followed by lupine, pigeon pea, adzuki bean, and soybean, and fell above the range in values for other pulses (Table 4). Bakoglu et al. (2009) found higher Fe content (136.88 ppm) and lower Zn content (14.48 ppm) in seeds of *O. fallax*. In an analysis of seeds from 20 sainfoin genotypes, Kaplan et al. (2019) found a significant effect of genotype on mineral content, with higher Fe content (113.7–277.7 ppm) and lower Zn content (26.03–52.39 ppm) than we found. Zinc and iron are two minerals of particular interest because of their critical role in human nutrition, especially in populations (e.g., pregnant women and children) vulnerable to malnutrition from deficiencies (Beal et al., 2017; Bruins et al., 2018; Dewey, 2013; Marangoni et al., 2016). Therefore, concerted efforts have been made in the last decade to improve the essential micronutrient content of cereal grains and legumes (Bouis et al., 2011; Jha & Warkentin, 2020; Kumar & Pandey, 2020; Qaim et al., 2007).

3.5 | Sainfoin phytic acid content higher than other pulses

Although sainfoin appears to be a potentially valuable source of protein and minerals, certain bioactive components like phytic acid can inhibit proteases, reducing protein degradation and subsequently digestion, and negatively impact mineral bioavailability (Chitra

TABLE 4 Total dietary fiber, iron, and zinc content reported for pulses, sainfoin, and soybean.

Crop	*N*	Total dietary fiber (g/100 g)	Iron (ppm)	Zinc (ppm)	Phytic acid (IP6) (mg/100 g)
Sainfoin	**15**	**48.96 ± 2.91 (45.79–54.34)**	**64.14 ± 6.38 (56.25–74.24)**	**61.63 ± 7.25 (54.78–79.05)**	**1790.89 ± 319.87 (1423.45–2288.24)**
Lupine[a]	6	39.76 ± 3.6 (35.77–46.6)	65.13 ± 18.57 (43.86–94.92)	53.65 ± 16.21 (30.7–77.22)	515.17 ± 127.76 (313–717)
Bambara groundnut[a]	1	31.8	30.03	21.35	275.6
Pigeon pea[a]	3	24.35 ± 0.75 (23.73–25.18)	63.83 ± 9.95 (57.03–75.25)	52.53 ± 17.1 (32.97–64.6)	544.48 ± 16.97 (525.36–557.76)
Common bean[a]	10	23.67 ± 1.87 (20.5–26.21)	80.37 ± 10.35 (65.63–99.23)	33.44 ± 2.35 (29.84–36.29)	856.62 ± 51.04 (772.2–937.2)
Broad bean[a]	2	22.05 ± 1.77 (20.81–23.3)	49.8 ± 11.9 (41.39–58.22)	33.34 ± 9.18 (26.85–39.83)	465.22 ± 24.44 (447.94–482.5)
Mungo bean[a]	2	21.97 ± 0.55 (21.58–22.35)	80.29 ± 1.77 (79.04–81.54)	34.43 ± 1.08 (33.67–35.19)	743.7 ± 0 (743.7–743.7)
Chickpea[a]	6	21.12 ± 4.71 (14.26–28)	74.03 ± 16.75 (48.96–94.71)	34.91 ± 3.55 (28.77–38.91)	605.56 ± 21.7 (590.15–645.86)
Lima bean[a]	1	21.04	71.51	30.49	657.36
Pea[a]	10	19.74 ± 5.6 (11.44–28.74)	52.06 ± 8.72 (38.83–66.82)	34.75 ± 4.01 (27.78–40.77)	501.42 ± 69.46 (427.68–582.12)
Hyacinth bean[a]	1	18.32	66.86	63.38	650
Mung bean[a]	3	17.07 ± 1.83 (15.27–18.92)	46.24 ± 2.37 (44.59–48.96)	22.41 ± 7.63 (17.98–31.22)	334 ± 0 (334–334)
Cowpea[a]	4	17.05 ± 6.91 (12.1–27.02)	59.85 ± 5.47 (52.74–65.14)	31.05 ± 7.83 (21.38–40.36)	687.87 ± 0 (687.87–687.87)
Lentil[a]	7	16.64 ± 2.38 (11.7–18.8)	84.43 ± 8.54 (72.06–97.3)	41.02 ± 5.33 (33.07–49.58)	388.14 ± 0.38 (388–389)
Moth bean[a]	2	16.46 ± 0 (16.46–16.46)	79.52 ± 0.22 (79.37–79.68)	43.78 ± 0.12 (43.7–43.87)	445.24 ± 0 (445.24–445.24)
Rice bean[a]	2	16.07 ± 1.45 (15.04–17.1)	67.65 ± 0.09 (67.59–67.72)	31.99 ± 0.04 (31.96–32.02)	280 ± 0 (280–280)
Adzuki bean[a]	1	14.67	51.56	56.39	
Soybean[b]	1	10.17	173.17	53.94	

Note: Phytic acid content reported for pulses and sainfoin. Values reported as mean value ± standard deviation (minimum–maximum), on an edible portion dry matter (EPDM) basis; rows sorted by total dietary fiber content; *N* = 6 for sainfoin phytic acid content; IP6 = inositol hexaphosphate.
[a]FAO (2017).
[b]USDA ARS Food Data Central.

et al., 1995; Sá et al., 2020; Sandberg, 2002). However, micronutrient bioavailability has been oversimplified by relying solely on estimates using phytic acid, and the beneficial role of phytic acid in human nutrition (e.g., antioxidant, antidiabetic, and antibacterial) has been overshadowed by these concerns (Kumar et al., 2021; Lopez et al., 2002; Schlemmer et al., 2009). Sainfoin had a considerably higher content of phytic acid compared with the pulses, which had mean values ranging from 334 mg/100 g EPDM (mung bean) to 856.62 mg/100 g EPDM (dry bean) (Table 4). Moreover, we observed the largest range in phytic acid content for sainfoin (864.79 mg/100 g sample EPDM), followed by lupine (404 mg/100 g sample EPDM), common bean (165 mg/100 g sample EPDM), and pea (154.44 mg/100 g sample EPDM) (Table 4). Within sainfoin, the variety Eski had the highest phytic acid content we observed (2288 mg/100 g sample EPDM), followed by Shoshone (1874 mg/100 g sample EPDM), Renumex (1725 mg/100 g sample EPDM), Remont (1582 mg/100 g sample EPDM), and Delaney (1503 mg/100 g sample EPDM) (Table S3). A wide range in variability for phytic acid has been shown for common bean (Akond et al., 2011), as well as for chickpea, common beans, broad bean, lentil, and pea (Shi et al., 2018). According to data reported by Biletska et al. (2020), soybean appears to have higher phytic acid content (2930 mg/100 g) than sainfoin. Chitra et al. (1995) also reported higher phytic acid content for soybean (3640 mg/100 g) and provided values for mung bean (1200 mg/100 g) and chickpea (960 mg/100 g). Furthermore, certain genotypes with low phytic acid have been identified within germplasm of chickpea, pigeon pea, urd bean, mung bean, and soybean (Chitra et al., 1995), chickpea (Bhagyawant et al., 2018), fava bean (Zehring et al., 2022), and common bean (Hummel et al., 2020). It is possible that low phytic acid genotypes could be identified as part of a strategy to manage levels within sainfoin seeds.

In addition to germplasm evaluation and selection, adequate processing techniques can mitigate the risk of bioactive components like phytic acid (Antoine et al., 2022; Singh et al., 2017; Yadav et al., 2019). Soaking reduces phytic acid content, in addition to total phenolic content, with total reduction depending on the pulse type and soaking conditions (Hall et al., 2017; Patterson et al., 2017; Tyler et al., 2017). Although decortication of pulses is also common, it has been shown to significantly increase phytate content in field peas because of phytic acid being primarily located in the cotyledons (Beal & Mehta, 1985; Wang et al., 2009). Conversely, removal of lentil seed coats, where most polyphenols are localized (Han & Baik, 2008), has been shown to reduce phytic acid and thus increase iron bioavailability despite a concurrent reduction in iron content (DellaValle et al., 2013). Cooking has been shown to significantly decrease both phytic acid and tannins in pulses (Beal & Mehta, 1985; Khalil & Mansour, 1995; Wang et al., 2008; Wang et al., 2009). For example, Thavarajah et al. (2009) saw a 99% reduction of phytic acid in Pardina lentils after cooking and more than 50% reduction in the three other lentil genotypes studied. Fermentation and germination, in addition to other methods that increase the activity of phytase enzymes that hydrolyze phytic acid, can improve the bioavailability of vital minerals like iron by reducing phytic acid content (Cook, 2005; Jha & Warkentin, 2020). Studies investigating various processing techniques will be required to quantify the extent of and develop recommendations for phytic acid reduction possible for sainfoin.

3.6 | Comparisons of sainfoin carbohydrates and carbohydrate components

Sainfoin, lupine, and soybean carbohydrate contents fell below the range of mean values for the pulses, which ranged from 66.84 g/100 g EPDM (broad bean) to 73.69 g/100 g EPDM (rice bean). As with the other proximates, the composition of the carbohydrate fraction is important to consider and more informative than the content alone. Total carbohydrates can be monosaccharides (ribose, glucose, galactose, and fructose), disaccharides (sucrose and maltose), oligosaccharides (mostly α-galactosides) and other polysaccharides, starch (including total starch, amylose, and amylopectin content), dietary fiber (including total, soluble, and insoluble fiber), and resistant starch (Cotacallapa-Sucapuca et al., 2021; Hall et al., 2017). We found sainfoin total starch content to range from 7.1 to 9.9 g/100 g EPDM with a mean value of 8.2 (sd = 0.8) and total sugar content to range from 4.9 to 6.3 g/100 g EPDM with a mean value of 5.7 (sd = 0.4). Of the sugars, sucrose had the highest mean value (5.1 g/100 g EPDM; sd = 0.4), followed by fructose (0.5 g/100 g EPDM; sd = 0.1) and glucose (0.1 g/100 g EPDM; sd = 0.1). Lactose and maltose values fell below the detection limit (<0.1 g/100 g sample) (data not shown). Compared with data reported in a review by Hall et al. (2017), the range we observed for total sugar content is comparable with most pulses, and the range in total starch is considerably lower than most pulses (lentil, 37%–59%; peas, 30%–49%; common bean, 18%–45%; chickpea, 30%–56%) and is most like lupine (1%–9%). Although low starch content may hinder the inclusion of sainfoin in extruded products, which traditionally rely on starch raw materials to produce highly consumed food products such as breakfast cereals and snacks (Cotacallapa-Sucapuca et al., 2021; van der Sman & Broeze, 2013), it may contribute to a lower glycemic index compared with other pulses.

The exceptional nutritional quality of legumes has historically played an important role in maintaining and improving human health. A growing body of evidence builds on this history and supports the contemporary role of pulses in preventing and treating chronic diseases, such as type II diabetes, heart disease, obesity, numerous cancers, and inflammatory disorders, in addition to contributing to gut health, via bioactive components (Clemente & Olias, 2017; Didinger et al., 2022; Jenkins et al., 2012; Polak et al., 2015; Rebello et al., 2014). Among these bioactive components, carbohydrates and proteins resistant to digestion might provide beneficial health impacts via gut health (Clemente & Olias, 2017; Hall et al., 2017; Zinöcker & Lindseth, 2018). In 1976, Trowell (1976) presented the hypothesis that dietary fiber was associated with human health benefits based on the research of Burkitt et al. (1972). The influence of dietary fiber on physiological effects that are linked to reduced risk for several chronic diseases has been extensively documented (Cho et al., 2013; Park et al., 2011; Reynolds et al., 2019). Sainfoin dietary fiber is composed mostly of insoluble fiber (37.9 g/100 g EPDM; sd = 1.9), compared with soluble fiber (6.6 g/100 g EPDM; sd = 0.9). We found a mean value for sainfoin total dietary fiber (TDF) content well above the range of mean values for pulses (Table 4), with lupine having the most similar content. TDF in pulses varies between 3% and 30% and is influenced by factors such as genotype, environment, management, seed maturation, processing conditions, and their interactions (Cotacallapa-Sucapuca et al., 2021; Hall et al., 2017). It is important to note that decortication of pulses reduces TDF in general, primarily through the reduction in insoluble dietary fiber located in the seed coat, and that total reduction varies by pulse (Hall et al., 2017). For example, Mubarak (2005) reported a reduction in TDF of 11% for mung bean, and Sudha et al. (1995) reported a reduction of 62% for horse gram. Given these trends, it is likely that sainfoin TDF content will be reduced following decortication, and the extent of any reduction will need to be quantified. Nevertheless, if these results are corroborated by additional studies, which are warranted, sainfoin may have higher dietary fiber content than any other pulse crop. Uses and benefits of sainfoin seed coats, where most of the dietary fiber may be located, could be like those shown for lupine (Malekipoor et al., 2022; Zhong et al., 2020).

Dietary fiber has been identified as a "nutrient of concern" in the United States since 2005 (McGuire, 2011; Quagliani & Felt-Gunderson, 2016). The prevalent "fiber gap" in the United States has been well documented (Jones, 2014; Mobley et al., 2014; Quagliani & Felt-Gunderson, 2016), where more than 90% of women and 97% of men do not meet recommended intakes for dietary fiber by a shortfall of approximately 50% (Thompson, 2021). In the same populations where the fiber gap exists, epidemic levels of obesity and comorbidities have been observed (Committee on Accelerating Progress in Obesity Prevention et al., 2012) and 74% of Americans have overweight or obesity (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020). Pulses are a valuable source of dietary fiber, containing two to three times more fiber per 100 g than other dietary staples (Chen et al., 2016), and present a compelling opportunity to serve a fundamental role in closing the fiber gap in the United States (Chen et al., 2016; Didinger & Thompson, 2021, 2022; Mitchell et al., 2009; Thompson, 2021). Despite the nutritional value of pulses, it has been observed that fewer than 8% of Americans consume pulses on any given day (Mitchell et al., 2009, 2021). In both the United States and Canada, those that do consume pulses exceed daily dietary fiber requirements (Mitchell et al., 2021; Mudryj et al., 2012). Moreover, their intake is likely underestimated, because the nutrient databases used were formed before a more accurate method of analysis for dietary fiber (AOAC 2011.25) was developed (Chen et al., 2016). Sainfoin could provide a higher fiber option compared with other pulse crops and could increase the feasibility of meeting or exceeding the recommended intake level while providing more protein as a perennial pulse.

3.7 | Correlation between sainfoin seed components

We define weak, moderate, and strong correlations based on correlation coefficient values between 0.00 and 0.39, 0.40 and 0.69, and 0.70 and 1.0, respectively. No significant correlations were found between sainfoin TSW and seed components (Table 5). This may have occurred because of narrow genetic diversity and a relatively small number of samples used in this study. In contrast, Tahir et al. (2011) reported significant correlations ($P < 0.05$) between seed weight and starch content ($r = 0.6$) and between seed weight and protein content ($r = -0.3$) in a study of 22 lentil genotypes grown in Saskatchewan, Canada. Additionally, in a study of desi and kabuli chickpeas also grown in Canada, Frimpong et al. (2009) reported the presence of significant correlations that varied across the two types. For example, seed weight had a positive correlation with starch in kabuli ($r = 0.16$, $P < 0.05$) and was not significant in desi.

We found moderate and strong negative correlations between protein content and ash, carbohydrates, fat, iron, and zinc, as well as between carbohydrates and TDF (Table 5). Fat had a moderate positive correlation with carbohydrates and zinc and a strong positive correlation with iron. Carbohydrates had a moderate and strong positive correlation with zinc and iron, respectively. Iron and zinc had a strong positive correlation. Although we did not find any significant correlations between total starch and the other seed components, Tahir et al. (2011) reported a significant negative correlation between starch and protein ($r = -0.5$) and Frimpong et al. (2009) reported negative correlations between starch and protein concentrations in desi ($r = -0.16$, $P = 0.05$) and kabuli ($r = -0.25$, $P = 0.01$) chickpea varieties. Across types and environments, Frimpong et al. (2009) found significant correlations between seed yield and protein ($r = -0.19$, $P < 0.01$) and starch ($r = 0.16$, $P < 0.01$). They did not find a significant correlation between seed weight and seed yield. In the context of soybean, Assefa et al. (2019) made the distinction that protein and oil concentration are relative measures, and when combined with seed yield, an absolute measure in the form of protein or oil yield is achieved. These results provide examples of how selecting for higher yielding varieties may influence pulse seed composition and how the relationships between yield components like seed weight should be considered. It will be most useful to quantify sainfoin seed components along with seed yield, to produce measures of component yields (e.g., protein yield and oil yield) that could support a breeding strategy to improve seed yield while simultaneously improving nutritional quality.

3.8 | PC analysis—Sainfoin components

PC analysis of the variables included in the correlation analysis revealed that PC1 and PC2 explained 66.5% of the total variance (Figure 2). For each PC, variables that had contributions greater than the threshold value (11.11%; expected value if the contributions were

TABLE 5 Pearson correlation coefficients between sainfoin seed components.

	TSW	Ash	Protein	Fat	Carbohydrates	Starch	TDF	Iron	Zinc	Phytic acid
TSW	1.00									
Ash	−0.49	1.00								
Protein	0.37	−0.68**	1.00							
Fat	−0.32	0.50	−0.65**	1.00						
Carbohydrates	−0.44	0.68**	−0.71**	0.52*	1.00					
Starch	0.27	−0.17	−0.23	−0.01	−0.19	1.00				
TDF	0.09	−0.36	0.33	−0.25	−0.55*	0.11	1.00			
Iron	0.02	0.58*	−0.64**	0.73**	0.70**	0.00	−0.37	1.00		
Zinc	−0.21	0.51	−0.69**	0.59*	0.67**	−0.09	−0.33	0.80***	1.00	
Phytic acid	−0.80**	0.88**	−0.91***	0.59	0.95***	0.26	−0.46	0.79*	0.80*	1

Abbreviations: TDF, total dietary fiber; TSW, thousand seed weight.
$^{***}P < 0.001$.
$^{**}P < 0.01$.
$^{*}P < 0.05$.

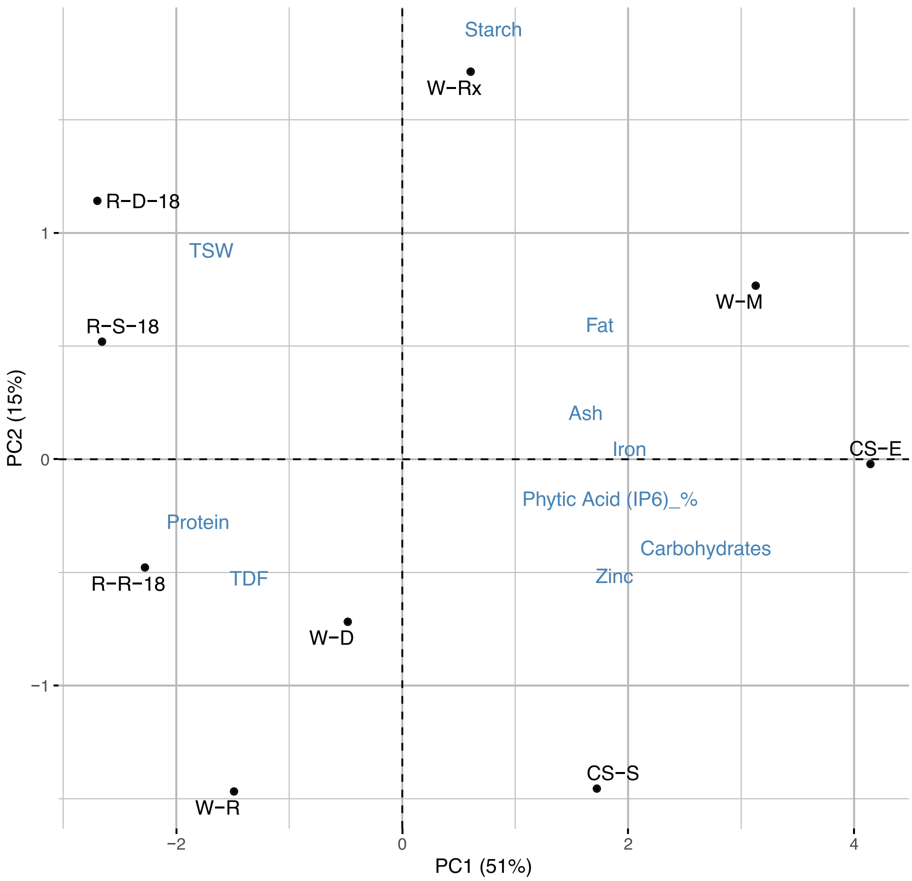

FIGURE 2 Biplot of principal components (PCs) 1 and 2 from PC analysis of sainfoin thousand seed weight (TSW) and seed components. Variables are represented by arrows, and individual samples are represented by filled circles, accompanied by sample identities. TDF, total dietary fiber.

uniform across variables) are reported in decreasing order. For PC1, carbohydrates, protein, iron, zinc, ash, and fat had contributions greater than the threshold (data not shown). For PC2, total starch and TSW had contributions greater than the threshold. This result is supported in part by coefficient of variation (CV) values being largest for zinc (11.5%) and iron (11.1%), followed by total starch (9.6%) and TSW (7.9%), with CV values being the smallest for TDF (4.7%), protein (4.0%), and carbohydrates (3.5%) (data not shown). Along the axis for PC1, samples in the positive direction (e.g., R-S-18, R-D-18, S-D-18, and R-R-18) are primarily differentiated by higher protein content compared with samples in the negative direction (CS-E and W-M) that have higher carbohydrate, iron, zinc ash, and fat content. Along the axis for PC1, samples are primarily differentiated by TSW and starch content, with those in the positive direction having higher values (e.g., CF-R-19 and MG-D-18). Using PCA to investigate pulse crop functional diversity, Guiguitant et al. (2020) reported PC1 (33% of total variance) associations with morphological traits (e.g., leaf traits) and to a lesser extent with seed diameter and weight, whereas PC2 (15% of total variance) had strong associations with seed traits such as TSW, seed diameter, and seed oil and protein content. Even with the limited number of samples analyzed, our results indicate that seed components, especially carbohydrates and protein, as well as TSW and starch, could drive variation among sainfoin samples.

Given the opportunistic sample of sainfoin seeds, representing different years, growers, and varieties, cautious inferences can be made regarding possible relationships and factors that influence the variation observed. A grouping of Remont (D) samples from three different growers in the direction of TSW and TDF could indicate that these varieties may be stable for these traits, despite possible differences in production and environment. A grouping of Rocky Mountain Remont (R) samples in the direction of protein content could indicate that this variety may have high protein content potential. However, one Rocky Mountain Remont sample by grower CF is in the direction of high TSW and starch content, which could indicate possible genotype-by-environment interactions with respect to these traits. Furthermore, several Shoshone (S) samples are located in the direction of higher protein content, and one sample from grower CS is in the direction of higher carbohydrates, and ash could also indicate the possibility of genotype-by-environment interactions. The tight grouping of Shoshone samples from grower PJ could indicate consistent production across years for this variety and this grower. In the direction of high zinc, iron, and fat content, three separate varieties, which do not occur elsewhere on the biplot because of a lack of replication, could provide an indication of distinctive mineral and fat content compared with the other varieties. Again, several caveats restrict the strength of these possible relationships.

3.9 | Research gaps and future directions

By addressing challenges and research gaps, abundant opportunities exist to support the development of sainfoin as a perennial pulse crop. Looking to major pulse crops reveals likely pathways forward. This includes research into genomics and genetics, agronomy, nutritional quality, post-harvest processing, and food science. However, certain aspects of a pulse crop with a perennial growth habit will require unique innovations to overcome specific challenges. These specific challenges include stand establishment and longevity, accumulation of pest and disease pressure, varying levels of seed maturity and presence of high moisture biomass at harvest, developing best practices for seed production and harvest along through appropriate infrastructure and protocols, and management strategies that balance the benefits from numerous possible end uses.

At the species level, further research into sainfoin seed composition is required to corroborate results for the components analyzed in this study, investigate components that were not analyzed, and identify any components that may not have been initially considered but warrant investigation as research advances. In this study, we analyzed several major seed components whose constituents require further characterization and quantification. For example, protein and oil content will be better understood when amino acid and fatty acid profiles are determined, as will starch content with the quantification of amylose and amylopectin ratios. Other components of interest not analyzed in this study include, but are not limited to, antioxidants and their activity, vitamins, minerals, polyphenols, resistant starch and oligosaccharides, and protein subunits. To ensure safety as a novel human food, careful and thorough investigation is required to identify any deleterious compounds and associated toxicology. These compounds could include non-protein amino acids, phytoestrogens, protease inhibitors, heavy metals, and lipoxygenases. Large variation among sainfoin accessions has been reported for foliar condensed tannin content (Wang et al., 2015) and phenolic content (Thill et al., 2012), and a preliminary study of sainfoin seeds detected phenolics associated with health benefits such as reduction of blood pressure, detoxification, and providing anticancer properties in humans (Wijekoon et al., 2021). Therefore, special attention should be given to these compounds. Although pending and ongoing studies are aimed at filling these gaps, future research agendas should certainly remain flexible to adapt to unanticipated and emergent challenges.

Below the species level, there is much to learn regarding the extent of variation related to seed components. Although we did not have sufficient replication to test differences among the sainfoin varieties in this study, future studies with robust sampling designs will be conducted with this goal in mind. Evaluation of diverse sainfoin germplasm, such as the approximately 500 accessions in the USDA National Plant Germplasm System's *Onobrychis* sp. collection, will further our understanding of how genetic diversity can support the improvement of sainfoin nutritional quality. Other sources of variation, such as environment and management practices, may independently influence seed composition and may also interact with variety or genotype to do so. Therefore, ongoing and pending studies, including a row spacing and variety trial, a larger multi-genotype, multi-year, and multi-environmental trial, and intercropping trials with other perennial legumes and grains (e.g., Kernza®), are aimed at systematically unraveling complex and multiplicative interactions. Ultimately, these studies, along with future ventures into nutritional phenomics, will reveal viable breeding strategies to improve agronomics (e.g., seed yield) and nutritional quality.

Processing methods and various traditional and contemporary uses represent an additional layer of complexity that could influence nutritional quality. Processing techniques, such as milling, soaking, decortication, germination, fermentation, and extrusion, may be valuable ways to improve nutritional quality, texture, and taste. Future studies should evaluate these techniques and quantify their impact on nutritional quality. These techniques could also impact sainfoin functionality in food products. The functionality and application of sainfoin seeds and products, such as flour and protein isolates or concentrates, will rely on the abundance of seed components, their composition, structure, and functionality. Studies will need to evaluate chemical and physical characteristics, including seed composition, flour pasting properties, water and oil holding capacity, water solubility and absorption, emulsification and foaming capacity and stability, and extrusion capabilities, as well as cooking quality and texture. Together, these indicators of functionality can then be used to determine suitability to various applications, such as use in soups and salads, baked goods, pastas, extruded products, beverages, and plant-based meat and dairy analogs. Ultimately, the extent of adoption and integration of sainfoin into human diets will rely on information gained through these research efforts, as they determine the range of potential uses and products, in addition to cost, convenience, consumer acceptance, and the perceived benefits to human health and the environment relative to other pulses and products.

4 | CONCLUSION

This study evaluated the nutritional quality of sainfoin seeds, which were opportunistically sampled from commercial seed lots in Montana and Idaho, USA. This study demonstrates a unique seed composition for sainfoin, with possible benefits for human health and potential as a novel pulse crop. We found sainfoin crude protein content to be comparable with soybean and lupine and higher than other pulses. Fat content was comparable with chickpea and lupine. Ash content fell within the range for pulses, whereas zinc content was above the range for pulses. Phytic acid content was considerably higher than pulses, although common processing techniques may reduce phytic acid content to reasonable levels. Sainfoin had considerably higher dietary fiber content, which may indicate the potential for sainfoin to help pulse consumers meet or exceed dietary requirements to close the "fiber gap." Future studies should corroborate these results, further characterize seed components, and evaluate seed samples from a wider genetic diversity, range of environments, and management practices. Additionally, common legume processing techniques, such as decortication, soaking, and cooking, may influence sainfoin nutritional quality and should be investigated. Ongoing and future research and breeding efforts will support the domestication and development of sainfoin as a potential novel perennial pulse crop, an effort being led by The Land Institute in collaboration with global partners.

AUTHOR CONTRIBUTIONS

Conceptualization: Brandon Schlautman, Evan B. Craine, Tessa E. Peters, Spencer Barriball, Muhammet Şakiroğlu. Methodology: Brandon Schlautman, Evan B. Craine. Writing—original draft preparation: Evan B. Craine. Writing—review and editing: Evan B. Craine, Muhammet Şakiroğlu, Tessa E. Peters, Spencer Barriball, Brandon Schlautman. Funding acquisition: Brandon Schlautman. Resources: Brandon Schlautman, Spencer Barriball. Supervision: Brandon Schlautman.

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CONFLICT OF INTEREST

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or nonfinancial interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

Data are available upon request and will be made publicly available upon publication through the Sainfoin Consortium, a Germinate database (https://sainfoinconsortium.org/#/home).

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