Food Bioscience 62 (2024) 105275

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Ultrasound-assisted Co-extraction and characterization of saponin-rich Co-extracts from fenugreek seeds (*Trigonella foenum-graecum* L.) and quinoa husk (*Chenopodium quinoa* Willd.) and their hydrolysates

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ARTICLE INFO

Keywords:
Co-extraction
Saponins
Sapogenins
Phenolic compounds
Microwave-assisted acid hydrolysis
Ultrasound-assisted extraction

ABSTRACT

The co-extraction of steroidal and triterpenoid saponins, followed by hydrolysis to produce sapogenin-rich extracts, offers an efficient strategy to enhance the multi-bioactive potential of extracts. The resulting chemical composition modifications, including the presence of other co-extracted or co-hydrolyzed compounds, must also be considered. In this study, ultrasound-assisted co-extraction of fenugreek seeds and quinoa husk using methanol and 50% aqueous methanol was conducted to obtain extracts and co-extracts with varying proportions of steroidal and triterpenoid saponins, in ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively. Aqueous methanol produced the highest extraction yields (15–25%) and saponin contents (40–70%) in the extracts and co-extracts. The conversion by microwave-assisted acid hydrolysis of saponins to sapogenins was more efficient for triterpenoid saponins, leading to a higher triterpenoid sapogenin content in all co-extracts, accounting for over 70% of the total sapogenins. The co-extracts also contained other bioactive compounds, such as phenolic compounds (3–4 g/100 g), with their content being favored by the presence of fenugreek. Hydrolysis altered the overall composition of the saponin-rich co-extracts, enriching the resulting hydrolyzed products in both types of sapogenins, along with fatty acids, carbonyls, non-protein nitrogen compounds and organic acids, while reducing monoglycerides, removing carbohydrates and significantly modifying the phenolic profile by eliminating many original phenolic compounds and generating new ones. Therefore, co-extraction and post-extraction hydrolysis of fenugreek and quinoa allowed the combination of triterpenoid and steroidal saponins or sapogenins into single products, along with other specific compounds from both sources.

1. Introduction

Edible seeds from fenugreek (*Trigonella foenum-graecum* L.) and quinoa (*Chenopodium quinoa* Willd.) have gained interest in recent years, not only for their culinary and nutritional properties, but also for their minor bioactive phytochemicals, mainly saponins, along with phenolic compounds, phytosterols, carotenoids, alkaloids and tocopherols, among many other constituents (Aasim et al., 2018; Hernández-Ledesma, 2019). Saponins are glycosides characterized by having a steroidal or triterpenoid aglycone and one or more sugar chains. Thus, according to their aglycone structures, saponins can be divided into two categories: steroidal and triterpenoid saponins (Güçlü-Üstündağ & Mazza, 2007; He et al., 2019). In this sense, fenugreek seeds are characterized by steroidal-type saponins, while quinoa represents a source of triterpenoid-type saponins, predominantly found in the husk. Sapogenins from fenugreek include gitogenin, yamogenin, diosgenin, tigogenin and neotigogenin, while from quinoa the major aglycones are oleanolic acid, hederagenin, phytolaccagenic acid and serjanic acid (Kuljanabhagavad & Wink, 2009; Aftab et al., 2021). Beyond their culinary and nutritional value, the extraction of saponins from these plants is of particular interest to the food and pharmaceutical industries, where they can be utilized for the development of functional foods,

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https://doi.org/10.1016/j.fbio.2024.105275
Received 1 August 2024; Received in revised form 1 October 2024; Accepted 9 October 2024
Available online 10 October 2024

nutraceuticals, and natural therapeutic agents.

Saponins have been extensively studied due to their bioactivity. These compounds have been reported to possess a wide range of biological activities, such as hypolipidemic, immunostimulatory, anticancer, anti-inflammatory, antibacterial and antiviral, among others (Rao & Gurfinkel, 2000; Francis et al., 2002; Gómez de Cedrón et al., 2020; Baky et al., 2022; Timilsena et al., 2023; Xie et al., 2023). Therefore, the extraction of saponins is crucial for maximizing the beneficial use of these compounds in product formulations, providing a sustainable alternative to synthetic products. Nevertheless, it is important to remark that most sapogenins possess improved bioactivity and bioavailability than their former saponins (Di Liberto et al., 2010; Herrera et al., 2019; Navarro del Hierro et al., 2021; Simurabiye et al., 2022). Furthermore, some sapogenins may show certain biological effects not exhibited by the former saponins (Uemura et al., 2011).

Regardless of their chemical form as saponins or sapogenins, their structural diversity is reflected in their different physicochemical and biological properties (Güçlü-Üstündağ & Mazza, 2007). In general, there are no natural sources in which steroidal and triterpenoid saponins occur simultaneously in similar proportions, the content of one type is usually negligible in relation to the other, which becomes the majority. For this reason, the co-extraction of saponins from different plant sources, such as fenugreek and quinoa, is particularly significant because it enables the obtention of enriched extracts with complementary or synergistic bioactivities. Fenugreek provides steroidal saponins, while quinoa offers triterpenoid saponins, and combining these could enhance the overall bioactivity and broaden their potential applications in health-promoting products.

Combination of extracts from multiple plants has been typically used in ethnomedicine to putatively offer more potent effects than extracts from single plants. Mensah et al. (2019) have shown the potential phytochemical synergy between extracts of *Strophanthus hispidus* and *Aframomum meleguta* in their anti-inflammatory effects *in vivo*. Moreover, synergistic effects on antioxidant, antifungal and anti-inflammatory activities were also observed when combining extracts from *Combretum molle* and *Xylopia aethiopica*, with the latter combination surpassing the effects of the control drug *in vivo* (Mensah et al., 2020). In addition, Karlapudi et al. (2018) showed that the combination of three extracts from *Terminalia chebula*, *Curcuma longa* and *Boswellia serrata* inhibited the enzymatic activity of cyclooxygenase-2 (COX-2) and 5- lipoxygenase (5-LOX) in humans more effectively than the individual extracts. However, the production costs of these extracts may be increased due to the need to produce individual extracts before creating the desired blends. For this reason, an interesting alternative would be the co-extraction, which allows for the direct obtention of a single extract from different sources in a single extraction process, reducing the costs of energy, raw materials and extraction solvents. As example, a higher bioactivity of extracts obtained from rosemary and marjoram by co-extraction has been observed recently regardless of the ratio of each type of plant used (Villanueva-Bermejo et al., 2024). Sharma et al. (2023) also obtained multibioactive extracts by co-extraction of turmeric and dried coconut. Concerning saponins, our recent findings indicate that the combined extraction of two plant sources of saponins, such as fenugreek (as source of steroidal saponins) and quinoa husk (as source of triterpenoid saponins), in different proportions, results in extracts that exhibit the bioactivities characteristic of both steroidal and triterpenoid saponins. The potential for each of the bioactivities studied was marked by the predominant type of saponin (Cantero-Bahillo et al., 2024). Therefore, the co-extraction process could modulate a range of desired bioactivities, enhance specific bioactivities and/or even reveal new bioactivities by adjusting the ratio of starting materials during co-extraction. This approach is not only scientifically relevant, but also has practical implications for the production of natural, bioactive ingredients to meet the growing demand for functional and therapeutic products across various industries. Although there is a growing number of studies on co-extracts, the literature is currently scarce and no references on neither co-extracts of saponins nor co-extracts of fenugreek and quinoa have been found beyond our recent study that validated the multibioactivity of these co-extracts (Cantero-Bahillo et al., 2024).

Regarding the production of saponin-rich extracts, one of the most common techniques used is ultrasound assisted extraction (UAE) (Tiwari, 2015; Wen et al., 2018; Jegal et al., 2019; Yu and Fan, 2021; Dastan et al., 2022; Yusoff et al., 2022). Water, alcohols such as methanol and ethanol, or water-alcohol mixtures have been widely used for extraction of saponins from plant matrices (Güçlü-Üstündağ & Mazza, 2007; Navarro del Hierro et al., 2018). Subsequently, considering the superior bioactivity and interest of sapogenins compared to saponins, it would be interesting to use downstream transformation strategies of saponin extracts for the release of the sapogenin fraction. This approach would result in extracts rich in sapogenins, which cannot be obtained directly since these substances either do not exist in seeds or are present in minimal concentrations (Colson et al., 2020; Herrera et al., 2019; Navarro del Hierro et al., 2022). In this context, the proposed strategy of co-extraction of saponins from different sources, combined with the subsequent transformation into sapogenin-rich extracts, represents an innovative approach to develop novel bioactive products. This method aims to achieve heightened levels of free triterpenoid and steroidal compounds simultaneously within one single product, a combination of molecules not naturally found together. Therefore, co-extraction followed by post-co-extraction transformation may offer an alternative means to generate multi-bioactive extracts, combining diverse bioactivities provided by both types of sapogenins into a single product. Regardless of the bioactivity, this method may also offer the possibility of combining a wide diversity of other molecules derived from the co-extracted sources within a single product. These additional molecules, such as phenolic compounds, may also contribute to the overall richness of the product in bioactive molecules.

Concerning the transformation of saponins into sapogenins, hydrolysis is a common method that may also offer a new range of natural or synthetic 'metabolites' with superior biological activity (Herrera et al., 2019; Teng et al., 2004). It involves removing the aglycone part of the sugar chain and can be carried out usually by chemical, enzymatic or microbial methods (He et al., 2019; Yang et al., 2023). One of the most common methods is acid hydrolysis, although the acidic conditions may negatively affect the structure of sapogenins, which is linked to their bioactivity (Herrera et al., 2019). Therefore, improving hydrolysis conditions is of great relevance. In recent years, microwave irradiation has gained interest, as it has been widely used in chemistry and is often combined with other hydrolysis methods due to its high heating efficiency, ability to reduce reaction time, and increase reaction rate (Wang et al., 2014; Zhu et al., 2006). In our recent work, we demonstrated that microwave assisted acid hydrolysis (MAAH) was an efficient alternative to conventional heating acid hydrolysis for saponins from a commercial saponin-rich fenugreek extract (Navarro del Hierro et al., 2022). Therefore, evaluating MAAH for transforming co-extracts rich in saponins to obtain co-extracts rich in corresponding sapogenins from fenugreek and other sources such as quinoa, would be an interesting alternative. This approach also allows for testing the potential of MAAH technology for the particular case of complex co-extracts.

The aims of this study were to evaluate the production of extracts rich in both steroidal and triterpenoid saponins, in a single product, by co-extraction of fenugreek seeds and quinoa husk, using UAE. Different extraction solvents were evaluated, as well as various combination ratios of fenugreek and quinoa. Additionally, the study aimed to produce extracts rich in both types of sapogenins through MAAH of the previously obtained co-extracts. Finally, selected extracts were characterized, considering the content of saponins and sapogenins as well as other co-extracted minor compounds of interest.

2. Materials and methods

2.1. Reagents and materials

Seeds of fenugreek were purchased from Plantafarm S.A. (Villanueva del Condado, León, Spain) and quinoa husk powder was kindly provided by Algodonera del Sur S.A. (Lebrija, Sevilla, Spain). Diosgenin, oleanolic acid, and N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) were from Sigma-Aldrich Chemie GMbH (Steinheim, Germany). Protodioscin, hederacoside C, sarsasapogenin, gitogenin and hederagenin were from Cymit Química S.L. (Barcelona, Spain). Hydrochloric acid (2 M) was from Merck KGaA (Darmstadt, Germany). Ethyl acetate, methanol and 95% hexane were from Macron (Gliwice, Poland).

2.2. Production of saponin-rich extracts and co-extracts

Seeds of fenugreek were ground in a knife mill (Grindomix GM200, Retsch, Haan, Germany) at 10,000 rpm for 1 min. The defatting of the samples (ground fenugreek seeds or quinoa husk powder) was carried out using a conventional solid-liquid extraction with hexane at a ratio of sample to solvent of 1:5 (w/v) in an Ultraturrax (IKA, Staufen, Germany) at 11,000 rpm for 5 min. The mixture was centrifuged at room temperature for 10 min at 3396×*g*. The supernatant was removed and the precipitate was defatted again following the same procedure. The defatted precipitates were completely dried for 20 min at 50 °C to remove any residual solvent.

In an initial assay, defatted fenugreek and quinoa powders, along with their 1:1 mixture (F50Q50) were tested to select the optimal solvent for extracting saponins. The solvents evaluated were methanol, 70% aqueous methanol and 50% aqueous methanol, following the UAE direct sonication method described by Navarro del Hierro et al. (2018). Then, the mixture was centrifuged for 15 min at 2683×*g*. The methanolic phase was collected and completely removed under vacuum until constant weight. Samples extracted with aqueous methanol were freeze-dried (LyoQuest, Telstar, Barcelona, Spain) after vacuum-removal of methanol.

Following the selection of the extraction solvent, defatted fenugreek and quinoa along with their mixtures were extracted at different proportions (100% fenugreek, F100; 90% fenugreek and 10% quinoa, F90Q10; 75% fenugreek and 25% quinoa, F75Q25; 50% fenugreek and 50% quinoa, F50Q50; and 100% quinoa, Q100) following the same UAE conditions as previously described.

The resulting extracts were stored at −20 °C until further use. Extractions were performed at least in triplicate for each sample and the extraction yield was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{weight of saponin-rich extract or co-extract}}{\text{weight of defatted fenugreek and/or quinoa powder}} \times 100$$

2.3. Production of sapogenin-rich extracts and co-extracts

The hydrolysis of the previously obtained saponin-rich extracts was performed according to Navarro del Hierro et al. (2022) by MAAH. Different times of hydrolysis were tested (10, 20 and 30 min) at a constant temperature (140 °C) to validate the optimal hydrolysis time. Hydrolysis were performed at least in duplicate, and the yield of hydrolyzed extract was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{weight of hydrolyzed (sapogenin-rich) extract or co-extract}}{\text{weight of initial saponin-rich extract or co-extract}} \times 100$$

2.4. Quantification of saponins by high performance liquid chromatography (HPLC)

All the saponin-rich extracts and co-extracts obtained were analyzed by a HPLC system (LC-2010C 3D Plus System, Shimadzu Corporation, Kyoto, Japan) with diode array detection for the quantitative determination of the saponins according to Herrera et al. (2019).

2.5. Quantification of sapogenins by gas chromatography mass-spectrometry (GC-MS)

The sapogenin content of sapogenin-rich extracts and co-extracts was characterized and quantitated by GC-MS according to Herrera et al. (2019), previous derivatization of the samples with BSTFA at 7 mg/mL (75 °C, 60 min). Identification of compounds was performed by the NIST MS Data library and by mass spectra according to literature (Herrera et al., 2019; Navarro del Hierro et al., 2020). The quantification of sapogenins was performed using calibration curves obtained from commercial standards whenever possible, prepared under identical conditions. For fenugreek sapogenins, the following commercial standards were used: diosgenin (for diosgenin, yamogenin, yucagenin and steroidal sapogenin acetate molecules), sarsasapogenin (for sarsasapogenin, smilagenin, tigogenin and neotigogenin) and gitogenin (for gitogenin and neogitogenin). Similarly, for quinoa sapogenins, the quantification was performed using oleanolic acid (for oleanolic acid and serjanic acid) and hederagenin (for hederagenin and phytolaccagenic acid) as commercial standards.

2.6. Characterization of other compounds in the co-extracts and co-hydrolyzed extracts

Other minor compounds co-existing in the saponin and sapogenin-rich extracts and co-extracts were analyzed following the same procedure described for sapogenins, prepared under identical conditions and using the NIST MS Data library for identification.

Furthermore, the determination of phenolics was conducted using an Agilent 1260 HPLC Infinity series system equipped with a photodiode-array detector (PAD) (Agilent Technologies Inc., Santa Clara, CA, USA). The chromatographic method and conditions used were established according to the protocol detailed in Villalva et al. (2018). Phenolic compounds were quantified employing standard calibration curves when available. The phenolic content was expressed as g compound per 100 g of extract.

2.7. Statistical analysis

Statistical analyses were performed by means of the general linear model procedure of SPSS 26.0 statistical package (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance. Differences were considered significant at $p \leq 0.05$. Post-hoc Tukey's tests were performed in order to establish significant differences. Pearson's correlation tests were conducted for additional analyses.

3. Results and discussion

3.1. Saponin-rich extracts and co-extracts

As an initial assay, different methanol-based extraction solvents were evaluated to identify the one that yielded the highest saponin contents and extraction yields, with particular emphasis on these values for the co-extract F50Q50. Extracts from fenugreek (F100) and quinoa (Q100) were also obtained by UAE separately, for comparative purposes. Methanol and aqueous methanol were used as solvents according to Shrestha and Baik (2012) and Dahmoune et al. (2021), among others, due to the higher extraction yields and saponin contents obtained compared to other solvents such as ethanol, especially when the

obtention of saponin-rich extracts is performed by ultrasound extraction (Herrera et al., 2019; Wang et al., 2022).

A first analysis was performed with the co-extract F50Q50 using methanol, 70% methanol and 50% methanol as solvents. As shown in Fig. 1, the extraction yield increased with the aqueous percentage of the solvent, although the saponin content remained around 60 g/100 g for both aqueous methanol solvents. Thus, the highest extraction yields (21 g/100 g) were obtained when 50% methanol was used. As our main criteria for selecting the extraction solvent is to achieve the highest total saponin content together with the highest yield, 50% methanol was used for fenugreek and quinoa extractions individually, together with 100% methanol, for comparative purposes. In the case of individual fenugreek and quinoa extracts, a higher saponin content was also observed together with a higher extraction yield when using 50% methanol compared to methanol, which also confirms better results when aqueous methanol is used. The higher yield for the methanol-water mixture could be explained by a higher solubilization of the saponins in this more polar medium due to their structure (Ligor et al., 2018). These results confirm that aqueous methanol is necessary for the efficient extraction of saponins from fenugreek and quinoa compared to pure methanol. Proportions of water above 50% were not tested, as previous studies have shown that the extraction of saponins is less effective when the water content in the solvent mixture is increased. In contrast, when an organic solvent with lower polarity than water, such as methanol, is predominant in the aqueous mixture, the extraction is enhanced due to the less polar nature of saponins (Khatoon et al., 2024). Therefore, 50% methanol was selected as the extraction solvent for subsequent assays in this study.

After selecting the extraction solvent, different combinations of fenugreek and quinoa were formulated for co-extraction in a second assay. For this purpose, the initial total saponin content determined for the extracts F100 and Q100, as well as the co-extract F50Q50 were considered (Fig. 1). Additionally, the specific proportion of triterpenoid and steroid saponins obtained for the F50Q50 extract was also determined. As shown in Table 1, this extract was mainly composed of triterpenoid saponins (74%) along with steroidal saponins (26%), as determined by HPLC based on our previous studies (Herrera et al., 2019). This composition was attributed to the higher saponin content contributed by quinoa (Q100, 72%) compared to fenugreek (F100, 40%), as shown in Fig. 1. Therefore, these data were used to design the formulation of different combinations to obtain co-extracts with the following theoretical compositions: 1) equivalent triterpenoid and steroidal saponins (50% of each type); 2) predominance of triterpenoid saponins (approximately 75% of total saponins); and 3) predominance of steroidal saponins (approximately 75% of total saponins). To reach this theoretical compositions (Table 1), the following proportions of defatted fenugreek and quinoa were mixed for extraction: 1) a mixture of fenugreek and quinoa in a ratio of 75:25 [w/w], respectively, to achieve approximately 50% of total saponins as steroidal and 50% as triterpenoid ('F75Q25'); 2) a mixture of fenugreek and quinoa in a ratio of 90:10 [w/w], respectively, to reach a predominance of steroidal saponins ('F90Q10'); and 3) a mixture of fenugreek and quinoa in a ratio of 50:50 [w/w], respectively, for a predominance of triterpenoid saponins ('F50Q50').

Table 1
Theoretical and real proportion (%) of steroidal and triterpenoid saponins respect to total saponins in the fenugreek and quinoa co-extracts.

Co-extract	Steroidal saponins (%)		Triterpenoid saponins (%)		Total saponins (g/100 g)	
	Theoretical	Real	Theoretical	Real	Theoretical	Real
F50Q50	25.4	26.1 ± 1.1	74.6	73.9 ± 1.1	56.0	62 ± 3.6
F75Q25	50.6	54.7 ± 0.7	49.4	45.3 ± 0.7	48.0	53.5 ± 3.7
F90Q10	75.4	69.7 ± 0.9	24.6	30.3 ± 0.9	43.2	49.7 ± 0.5

After performing the different co-extractions and analysis in specific saponins, the contents in total steroidal and triterpenoid saponins were determined and the proportion of each group of saponin estimated. As shown in Table 1, the real obtained proportions closely matched the estimated theorical values for most cases. The minor differences may be due to the variability of the extraction method itself. Nevertheless, focusing on the total saponin value, in all cases the real value seemed to exceed the theoretical value, which could suggest the existence of some mode of synergies during the UAE co-extraction that favors the extraction of saponins, regardless of the type of saponin.

The actual content of steroidal and triterpenoid saponins in each extract and co-extract, along with their respective extraction yields, were detailed in Fig. 2. Regarding the total saponin content (Fig. 2), there were no significant differences between F90Q10 and F75Q25. However, higher quinoa content in all co-extracts resulted in increased total saponin content, with F50Q50 having the highest total saponin content. As expected, increasing the fenugreek or quinoa content during extraction led to significant increases in steroidal or triterpenoid saponins, respectively, in all co-extracts.

The extraction yield increased along with the quinoa content in the co-extracts (Fig. 2), possibly due to its naturally high saponin content and, consequently, the high affinity between these molecules and the solvent used, which favors their extraction. In addition, a strong positive

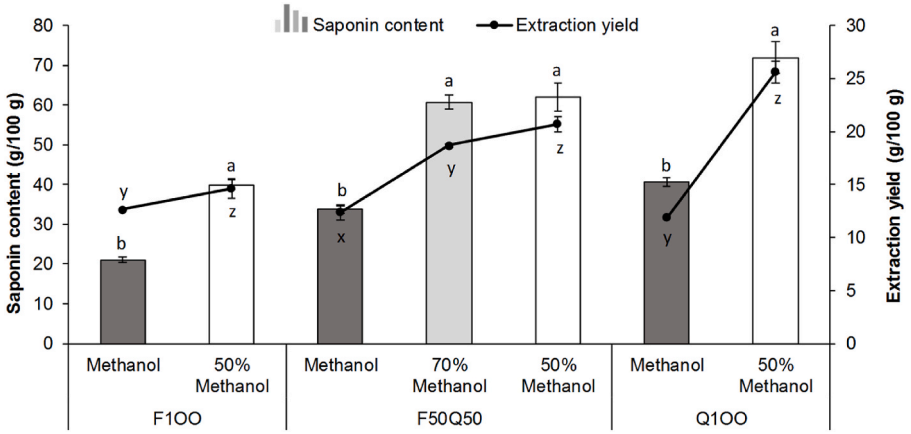

Fig. 1. Total saponin content (g/100 g) and extraction yield (g/100 g) of the extracts and co-extracts from fenugreek (F) and quinoa (Q) obtained by UAE using methanol or aqueous methanol as solvents. Mean values with different letters within each extract (a-b, total saponin content; x-z, extraction yield) are significantly different ($p \leq 0.05$).

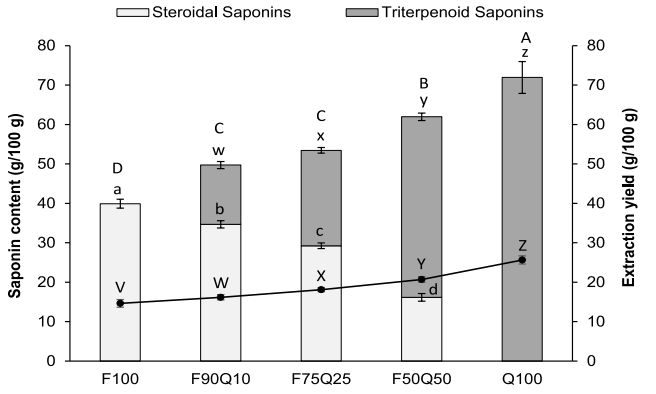

Fig. 2. Saponin content and extraction yield (g/100 g) of extracts and co-extracts from fenugreek (F) and quinoa (Q) at varying proportions obtained by UAE. Mean values with different letters (a-d, steroidal saponin content; w-z, triterpenoid saponin content; A-D, total saponin content; V-Z, extraction yield) are significantly different ($p \leq 0.05$).

correlation was observed between the extraction yield and the total saponin content (r = 0.936; $p < 0.001$). This outcome is of great interest, as it could allow for approximate predictions of the total saponin contents of extracts and co-extracts based on their extraction yields, regardless of the source (quinoa or fenugreek), their combinations for co-extraction, and the type of saponin (triterpenoid or steroidal).

The obtained results also demonstrate the possibility of obtaining extracts with tunable contents of steroidal and triterpenoid saponins simultaneously, which does not occur in significant proportions in nature. Extracts obtained from fenugreek, rich in steroidal saponins, are notable in the literature for their hypolipidemic and antidiabetic effects (Khatoon et al., 2024), while extracts rich in triterpenoid saponins from quinoa have been widely documented for their anti-inflammatory effects (Yao et al., 2014). For the co-extracts resulting from the combination of both types of saponins, we have already shown in a previous study that they possess anti-inflammatory, hypolipidemic and antioxidant properties, influenced by the predominant type of saponin in the co-extract. In his sense, the pancreatic lipase inhibition, the anti-inflammatory and antioxidant activities and the reduction of cholesterol bioaccessibility increased along with the fenugreek content (Cantero-Bahillo et al., 2024).

3.2. Sapogenin-rich extracts and co-extracts

3.2.1. Acid hydrolysis time by MAAH

All five saponin-rich extracts and co-extracts were subjected to MAAH to obtain sapogenin-rich products. The MAAH conditions were stablished based on previous results by Navarro del Hierro et al. (2022) for a commercial fenugreek extract rich in saponins, identifying 140 °C as the optimal hydrolysis temperature. Taking these MAAH conditions as reference, but considering that the co-extracts were based on fenugreek and quinoa and not just fenugreek as Navarro del Hierro et al. (2022) did, a kinetic study was carried out to evaluate the most suitable reaction time for these complex mixtures of saponins. As shown in Fig. 3, the maximum time tested at 30 min allowed obtaining the highest yield of hydrolyzed extracts together with the highest sapogenin content of all hydrolyzed extracts and co-extracts (Fig. 3A & B).

Thus, HF75Q25 and HF50Q50 showed the highest yields of the co-extracts (25–30%), with no significant differences with HQ100, which had a yield close to 35%. HF90Q10 showed the lowest yields of the co-extracts (around 20%), similar to HF100. Regarding the total saponin content at 30 min of hydrolysis, HF50Q50 showed the highest value (around 40 g/100 g) among the co-extracts, with no significant differences from HQ100, while HF75Q25 and HF90Q10 showed the lowest

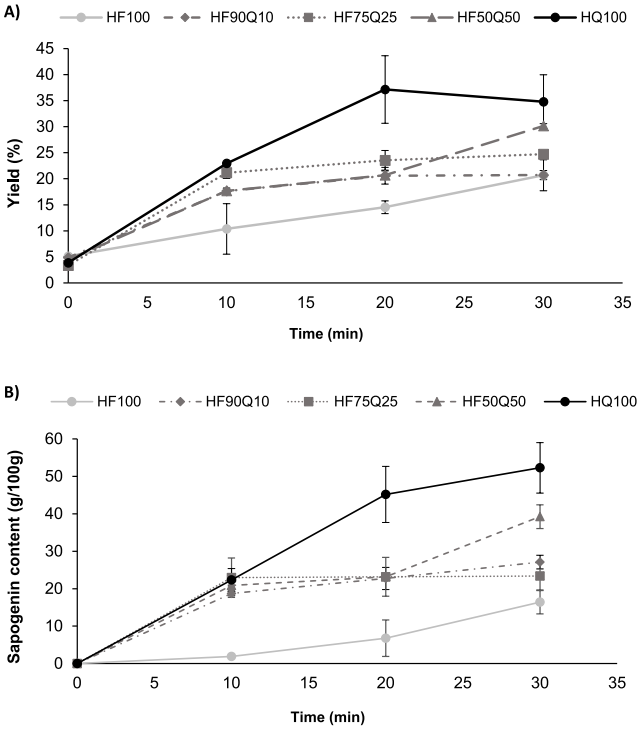

Fig. 3. A) Extraction yield (%) and B) sapogenin content (g/100 g) of hydrolyzed extracts and co-extracts obtained by MAAH from fenugreek (F) and quinoa (Q) saponin-rich extracts and co-extracts at different times of hydrolysis.

total content among the co-extracts (24–27 g/100 g), similar to HF100 (Fig. 3B). The yield of hydrolysis of the extracts at 30 min was positively correlated with the total sapogenin content (r = 0.885; $p < 0.001$). Therefore, 30 min was selected as the hydrolysis time, as longer durations were related to a decrease in both the hydrolysis yield and the sapogenin content (Navarro del Hierro et al., 2022). Additionally, in this study, yield and total sapogenin content remained stable for co-extracts HF75Q25 and HF90Q10, or even decreased in yield for HQ100.

3.2.2. Sapogenin profile of hydrolyzed extracts and co-extracts

A characterization of the extracts and co-extracts obtained at the final hydrolysis time was carried out in relation to the type of sapogenin that constituted them. As shown in Fig. 4, HQ100 extract showed the highest content in sapogenins (52 g/100 g), while HF100 the lowest (16

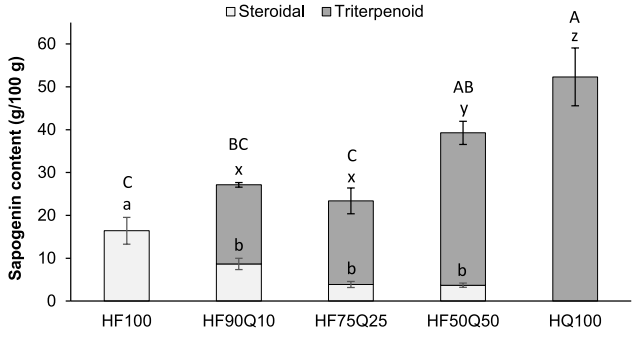

Fig. 4. Steroidal, triterpenoid and total sapogenin content (g/100 g) of hydrolyzed extracts and co-extracts from fenugreek (F) and quinoa (Q) at varying proportions obtained by MAAH at 30 min of hydrolysis. Mean values with different letters (a-b, steroidal sapogenin content; x-z, triterpenoid sapogenin content; A-C, total sapogenin content) are significantly different ($p \leq 0.05$).

g/100 g). Each of them contained only either triterpenoid or steroidal sapogenins, respectively, as observed previously for the saponin-rich extracts. Likewise, an increase in triterpenoid and/or steroidal sapogenins was found in the co-extracts as the proportions of quinoa or fenugreek increased, respectively. In the hydrolyzed co-extracts, HF50Q50 exhibited the highest total sapogenin content (39.3 g/100 g), which was significantly higher ($p = 0.044$) than that from HF75Q25 (23.4 g/100 g), and showed no significant differences from HF90Q10 (27.1 g/100 g). There were also no significant differences between the hydrolyzed co-extracts HF90Q10 and HF75Q25, although a gradual increase in the total sapogenin content would have been expected with increasing proportions of quinoa in the co-extracts, given that the conversion of triterpenoid saponins to sapogenins was higher than that of steroidal saponins from fenugreek.

The fact that a gradual increase in the total sapogenin content was not observed along with the proportion of quinoa in the co-extracts could be attributed to how hydrolysis affected each co-extract individually.

Focusing on the type of sapogenin (steroidal or triterpenoid) present in the hydrolyzed co-extracts (Fig. 4), no differences were observed in the content of steroidal sapogenins between any of them, with values ranging between 3 and 8 g/100 g. However, differences were noted in the triterpenoid sapogenin content in the hydrolyzed co-extracts. HF90Q10 and HF75Q25, with triterpenoid contents between 18 and 20 g/100 g, differed from HF50Q50, which had a content of 35.6 g/100 g.

All co-extracts were formed to a greater extent by triterpenoid sapogenins. Thus, co-extracts HF90Q10 and HF75Q25 presented around 70–85% of triterpenoid sapogenins, while HF50Q50 consisted of 90%. The percentage of steroidal sapogenins was very small in the case of co-extracts HF75Q25 and HF50Q50 (around 10–15%), showing no significant differences with HF90Q10 (30%). The fact that this last co-extract, formed mainly by fenugreek, presented a higher content of triterpenoid sapogenins with respect to steroidal sapogenins may be due to a higher conversion of triterpenoid saponins into sapogenins, as well as to a higher degradation of steroidal sapogenins during the hydrolysis process. Such degradation may be conditioned by temperature and hydrolysis time, among other factors (Herrera et al., 2019).

For a deeper characterization of sapogenins, the individual identified and quantified steroidal and triterpenoid sapogenins of the hydrolyzed extracts and co-extracts were detailed in Table 2. Focusing on steroidal sapogenins, diosgenin was the major aglycone for all the hydrolyzed fenugreek extracts, as already documented (Bogdanovic et al., 2020; Herrera et al., 2019; Navarro del Hierro et al., 2020), reaching a maximum content of 5 g/100 g for the HF100 extract. The next most abundant aglycone in the hydrolyzed fenugreek extracts was diosgenin's epimer yamogenin, followed by tigogenin in HF100 and HF50Q50, and gitogenin in the co-extracts HF9010 and HF75Q25. Neotigogenin and sarsasapogenin had similar values in HF75Q25 and HF50Q50. Finally, smilagenin, neogitogenin and yuccagenin were the least concentrated. As expected, the content of each steroidal sapogenin decreased in the hydrolyzed co-extracts as the proportion of fenugreek decreased. Additionally, two spirostadienes were identified in all hydrolyzed extracts containing fenugreek, which are artefacts derived from the dehydration reactions of diosgenin and yamogenin in the presence of hydrochloric acid (Bedour et al., 1964). The content of these artefacts in the hydrolyzed co-extracts decreased along with the proportion of fenugreek and, consequently, the aglycones involved in their formation, decreased.

Regarding triterpenoid sapogenins, the four characteristic sapogenins of quinoa were identified: oleanolic acid, hederagenin, serjanic acid and phytolaccagenic acid (Medina-Meza et al., 2016; Navarro del Hierro et al., 2020). Their total content decreased in the hydrolyzed co-extracts as the proportion of quinoa decreased. Oleanolic acid, followed by hederagenin, were the major aglycones for extract HQ100 and co-extract HF50Q50, although there was a significant decrease in oleanolic acid for the hydrolyzed co-extract ($p = 0.026$). Medina-Meza et al. (2016) analyzed 16 varieties of quinoa with phytolaccagenic acid being the predominant aglycone in all of them, while Navarro del Hierro et al. (2020) determined that serjanic was the predominant one. The variations between the two studies and the present one may be due to the starting material as well as to the method and conditions of hydrolysis and extraction. In HQ100, oleanolic acid and hederagenin were followed by phytolaccagenic and serjanic acids. However, hederagenin and phytolaccagenic acid were the major aglycones in co-extracts HF90Q10 and HF75Q25, showing a large decrease in oleanolic acid content, which dropped by about 25% compared to HQ100. Similar behaviors were observed regarding the variation of the third most abundant steroidal sapogenin (tigogenin or gitogenin) in the hydrolyzed fenugreek co-extracts. This suggests that hydrolysis affects each sapogenin individually, indicating different stabilities or varying magnitudes of hydrolysis for each sapogenin.

Therefore, as observed for the saponin-rich co-extracts, these results demonstrate the potential for obtaining extracts with tunable contents of steroidal and triterpenoid sapogenins, which do not naturally co-exist in significant proportions. These products could be utilized in future bioactivity studies, in which the type and quantity of sapogenins, as was well as their potential synergies may influence the outcomes. For instance, HF90Q10 co-extract, which contains 9% steroidal sapogenins and 18% triterpenoid sapogenins, with 3% in the form of diosgenin and 5% as oleanolic acid—both of which are well-known bioactive molecules—would be particularly interesting as a multibioactive extract. This single product would combine the diverse bioactivities provided by

Table 2
Individual sapogenins identified and quantified (g/100 g) in the hydrolyzed extracts and co-extracts of fenugreek (F) and quinoa (Q) at varying proportions.

Rt (min)	Sapogenins	HF100	HF90Q10	HF75Q25	HF50Q50	HQ100
20.49	3,5-Spirostadiene derivative	2.91 ± 0.25^{a}	1.12 ± 0.05^{b}	0.57 ± 0.09^{c}	0.51 ± 0.07^{c}	n.a.
20.53	3,5-Spirostadiene derivative	1.34 ± 0.04^{a}	0.59 ± 0.06^{b}	0.33 ± 0.03^{c}	0.38 ± 0.06^{c}	n.a.
21.28	Smilagenin	0.47 ± 0.10^{a}	0.23 ± 0.04^{b}	0.10 ± 0.02^{b}	0.09 ± 0.01^{b}	n.a.
21.39	Sarsasapogenin	0.73 ± 0.19^{a}	0.38 ± 0.08^{ab}	0.16 ± 0.03^{b}	0.16 ± 0.02^{b}	n.a.
21.80	Diosgenin	5.24 ± 1.56^{a}	2.92 ± 0.52^{ab}	1.20 ± 0.25^{b}	1.21 ± 0.15^{b}	n.a.
21.89	Tigogenin	1.22 ± 0.19^{a}	0.65 ± 0.10^{b}	0.29 ± 0.06^{b}	0.29 ± 0.02^{b}	n.a.
21.93	Yamogenin	2.23 ± 0.84^{a}	1.29 ± 0.22^{a}	0.53 ± 0.10^{a}	0.50 ± 0.08^{a}	n.a.
22.01	Neotigogenin	0.86 ± 0.16^{a}	0.41 ± 0.07^{b}	0.18 ± 0.05^{b}	0.17 ± 0.02^{b}	n.a.
22.97	Yuccagenin	0.14 ± 0.04^{a}	0.12 ± 0.02^{ab}	0.05 ± 0.01^{ab}	0.04 ± 0.01^{b}	n.a.
23.12	Gitogenin	0.93 ± 0.19^{a}	0.72 ± 0.13^{ab}	0.34 ± 0.05^{b}	0.28 ± 0.03^{b}	n.a.
23.29	Neogitogenin	0.33 ± 0.11^{a}	0.24 ± 0.04^{a}	0.12 ± 0.01^{a}	0.09 ± 0.01^{a}	n.a.
23.97	Oleanolic acid	n.a.	5.01 ± 0.39^{c}	5.75 ± 1.96^{c}	18.16 ± 1.49^{b}	30.67 ± 5.31^{a}
24.54	Hederagenin	n.a.	6.64 ± 0.04^{b}	6.78 ± 0.56^{b}	8.83 ± 0.73^{ab}	11.72 ± 1.49^{a}
26.11	Serjanic acid	n.a.	0.32 ± 0.03^{b}	0.42 ± 0.09^{b}	1.06 ± 0.00^{a}	1.47 ± 0.27^{a}
27.43	Phytolaccagenic acid	n.a.	6.48 ± 0.07^{a}	6.57 ± 0.38^{a}	7.51 ± 0.47^{a}	8.44 ± 1.11^{a}

a-c Different superscript letters within a line denotes statistically significant differences ($p \leq 0.05$).
n.a. = not applicable; the compound is not found in the extract.

both types of sapogenins. In this sense, we have recently demonstrated that post-extraction processes, such as acid hydrolysis, can modulate and even enhance the bioactive potential of fenugreek and quinoa co-extracts (Cantero-Bahillo et al., 2024). This approach results in extracts with higher antioxidant and hypolipidemic activities. Additionally, we observed that the anti-inflammatory activity, which was positively influenced by the fenugreek content in the saponin-rich extracts, improved further as the quinoa content in the sapogenin-rich extracts increased (Cantero-Bahillo et al., 2024).

Moreover, an additional advantage of co-extraction and hydrolysis treatments post-co-extraction is the reduction of costs, both in terms of energy and solvents, as well as raw materials. Conducting individual extractions and hydrolysis of each raw material and then combining the desired extracts would increase costs.

3.3. Characterization of other compounds in the co-extracts and co-hydrolyzed extracts

Considering that the saponin-rich and sapogenin-rich extracts from fenugreek and quinoa, both individually and in co-extracts (75% fenugreek and 25% quinoa), were shown to be the most bioactive in our previous study (Cantero-Bahillo et al., 2024), an exhaustive characterization of these samples was performed. The goal was to observe similarities and differences between the samples and to study how hydrolysis affects their composition. The characterization of the extracts by GC-MS was carried out after formation of trimethylsilyl derivatives for the less volatile compounds containing hydroxyl and carboxyl functional groups. This method allowed the identification of 24 compounds in F100, 31 compounds in F75Q25 and 35 compounds in Q100 for the saponin-rich extracts. However, hydrolysis affected each of the extracts differently: the number of compounds increased in fenugreek (26 compounds in HF100), remained constant in the co-extract HF75Q25, and decreased in quinoa (30 compounds in the HQ100). This count does not include the characteristic sapogenins of fenugreek and quinoa present in the extracts and co-extract described in the previous sections. Notably, most compounds in the saponin-rich extracts differed from those in the sapogenin-rich extracts.

Compounds were categorized into 12 subgroups based on their major chemical family (Table 3). Regarding the saponin-rich extracts, all contained a major group of lipids, especially monoglycerides, followed by sugars and organic acids. In general, co-extract F75Q25 showed an intermediate composition between F100 and Q100, due to the joint presence of specific compounds from fenugreek and quinoa. All saponin-rich extracts contained the same lipid compounds. The content of sugars, as well as that of acids, appeared to be linked to a higher extent to the presence of quinoa. In this regard, glycerol was the only sugar alcohol present in F100, and its presence was also observed, to a greater extent, in F75Q25 and Q100. As for organic acids, malic acid and citric acid were the only ones present in all saponin-rich extracts, and its content tended to be slightly higher in the co-extract F75Q25. Similarly, F75Q25 showed a higher overall content of total nitrogen compounds, especially amino acids and derivatives, resulting from the joint presence of specific nitrogen compounds of F100 and Q100. However, as for non-protein nitrogen compounds, only a guanosine derivative was determined for Q100 and F75Q25. Terpenes were related to the presence of fenugreek, with a single compound, a pregn-5-ene derivative (steroid subgroup), detected in both F100 and F75Q25.

Regarding the sapogenin-rich extracts, as mentioned above for the saponin-rich extracts, the hydrolyzed co-extract HF75Q25 consisted of both HF100 and HQ100-specific compounds, although some of the specific compounds were missing in the co-extract. As shown in Table 3, a complete absence of carbohydrates and a marked increase in acids, especially organic acids, were observed, which were favored by the presence of fenugreek. In general, the sapogenin-rich extracts consisted mainly of terpenes, which include the steroid and triterpenoid subgroups corresponding to the sapogenins characteristic of each of the sources studied (Table 2). Organic acids were the second most abundant group in HF100 and HF75Q25 followed by fatty acids and derivatives, whereas in HQ100 fatty acids and derivatives were the second most abundant subgroup, followed by organic acids.

Within the lipids group, the appearance of an α-linolenic derivative and a glycerol monostearate derivative was observed in all the extracts, both products could derive from the hydrolysis of residual glycerides present in the extracts rich in saponins. As for organic acids, there was an increase in the number of compounds, as well as a large increase in the content from the derivatives of malic and citric acids, already present in the saponin-rich extracts. As for nitrogen compounds, all sapogenin-rich extracts were composed mostly of non-protein compounds, with the presence of the amino acid cyclopentylalanine only observed in the case of HQ100. In addition, all sapogenin-rich extracts presented a new family, the carbonyl compounds, due to the presence of an unidentified cyclic ketone. Its presence may be due to a decarboxylation of carboxylic acids or β-ketoacids (Punekar, 2018), such as 5-methyl-2-furancarboxaldehyde present in HF100 and HF75Q25, as well as a derivative of 5-methyl-2-furancarboxaldehyde, present only in HQ100, and xanthurenic acid, present in HQ100 and HF75Q25.

In addition, the process of co-extraction enables to obtain extracts with a composition that is challenging to encounter in nature. This composition may be linked to the bioactivity exhibited by the co-extract, as it is conceivable that this bioactivity depends on both the inherent bioactive properties of saponins or sapogenins *per se*, as well as on the remaining compounds present in the extract. Moreover, the hydrolysis process allows the appearance of some compounds of great bioactive interest such as oleamide, a non-protein nitrogen compound present in HF100 and HF75Q25, which is known for its antinociceptive properties, among others, and has been previously documented by Chatterjee et al. (2010) as the main fatty acid amide of fenugreek.

Furthermore, although fenugreek and quinoa are not typically characterized as rich sources of phenolic compounds, their analysis is of great interest due to the implications it may have on their overall bioactivity. Additionally, it is noteworthy to examine the effect of hydrolysis on these compounds when aiming to obtain extracts rich in saponins. Indeed, the strategies for the hydrolysis of complex phenolic compounds, aimed at releasing their aglycones with enhanced bioactivity, currently represent a field of great research interest (Ebrahimi et al., 2024). Initially, saponin-rich extracts and co-extracts were analyzed to determine the initial phenolic content of the extracts. As shown in Table 4, the analysis revealed the presence of phenolic acids and their derivatives, as well as flavonoids and their glycosides. These compounds were source-specific, since fenugreek-derived compounds were absent in quinoa and *vice versa*.

The F100 extract, consisting only of fenugreek, contained primarily apigenin derivatives, homoorientin and salvianolic acid, among others, with a total phenolic content of 5 g/100 g of extract. In contrast, the Q100 extract, consisting only of quinoa, contained mostly quercetin derivatives, catechin and *p*-coumaric acid, among others, with a much lower total phenolic content of approximately 0.8 g/100 g of extract. These phenolic compounds align with those reported by other authors (Benayad et al., 2014; Gómez-Caravaca et al., 2014; Hemalatha et al., 2016; Król-Kogus et al., 2014), although the total contents varied, likely due to the differences in raw material and extraction methods, as this study used a saponin-focused extraction rather than one optimized for phenolic compounds. The co-extracts contained phenolic compounds from both sources, with specific content varying according to the fenugreek or quinoa proportion. Their total content ranged from 3 to 4 g/100 g extract. In addition, it was considered interesting to observe how the hydrolysis process affected the phenolic composition (Table 4). This analysis was focused on the hydrolyzed extract from F100 (HF100) due to the highest initial phenolic content of F100 among all five extracts and co-extracts. As shown in Table 4, most phenolic compounds disappeared during hydrolysis and/or transformed into new compounds such as hydroquinone derivatives, free luteolin and apigenin. In this

Table 3
GC-MS Characterization Profile (Total Area Counts x 10^4) of Compounds Identified in Saponin and Sapogenin Rich Extracts and Co-Extracts from Fenugreek (F) and Quinoa (Q) at Varying Proportions.

R_t	Compound	F100	HF100	F75Q25	HF75Q25	Q100	HQ100
LIPIDS							
	Fatty acids and derivatives	**54.04 ± 8.04**	**370.71 ± 23.67**	**58.93 ± 12.55**	**334.50 ± 37.15**	**65.00 ± 10.94**	**554.10 ± 40.20**
8646	Fatty acid n.i.	-	-	-	-	-	49.36 ± 23.40
15,112	Palmitic acid	2.03 ± 0.14	81.17 ± 16.72	4.92 ± 3.26	66.95 ± 8.18	3.51 ± 2.53	91.02 ± 0.52
16,115	Linoleic acid derivative	1.11 ± 0.28	126.89 ± 13.10	1.34 ± 0.01	177.46 ± 15.46	5.02 ± 4.00	289.58 ± 51.25
16,141	Oleic acid derivative	3.09 ± 0.99	-	1.65 ± 0.01	-	7.53 ± 6.56	-
16,147	α-Linolenic acid derivative	-	91.14 ± 4.68	-	90.10 ± 13.50	-	124.13 ± 11.83
16,28	Stearic acid derivative	1.37 ± 0.24	71.51 ± 24.73	1.40 ± 0.03	-	1.24 ± 0.74	-
17,176	Myristic acid derivative	1.24 ± 0.02	-	1.99 ± 0.78	-	1.47 ± 0.16	-
19,097	Octadecanoic acid derivative	45.21 ± 6.37	-	47.63 ± 1.10	-	46.23 ± 3.05	-
	Monoglycerides	**97.95 ± 26.01**	**23.45 ± 0.01**	**115.86 ± 14.34**	**8.31 ± 0.95**	**77.90 ± 7.30**	**15.84 ± 3.04**
17,997	2-Monopalmitin	21.89 ± 8.82	-	24.92 ± 2.97	-	4.48 ± 3.82	-
18,17	1-Monopalmitin	57.67 ± 8.98	-	73.28 ± 8.29	-	70.09 ± 0.35	-
18,171	1-Monopalmitin derivative	-	11.52 ± 0.78	-	4.22 ± 0.10	-	7.55 ± 1.80
18,931	2-Monoestearin derivative	18.39 ± 8.21	-	17.66 ± 3.08	-	3.33 ± 3.12	-
19,099	Glycerol monostearate derivative	-	11.92 ± 0.79	-	4.09 ± 0.85	-	8.29 ± 1.23
NITROGEN COMPOUNDS							
	Amino acids and derivatives	**5.70 ± 0.83**	**-**	**9.07 ± 1.56**	**-**	**3.07 ± 1.01**	**18.14 ± 9.62**
9171	Cyclopentylalanine	-	-	-	-	-	18.14 ± 9.62
11,407	L-Glutamic acid derivative	4.76 ± 0.55	-	7.47 ± 1.30	-	-	-
11,522	L-5-Oxoproline	0.94 ± 0.28	-	1.60 ± 0.26	-	2.22 ± 0.72	-
12,291	L-Glutamic acid derivative	-	-	-	-	0.85 ± 0.29	-
	Non-proteic compounds	**-**	**140.77 ± 18.88**	**1.93 ± 0.83**	**82.44 ± 17.57**	**6.02 ± 0.02**	**145.68 ± 11.35**
9271	4-Fluoro-2-methyl-1H-indol-5-amine	–	18.82 ± 1.95	–	15.38 ± 0.73	–	9.13 ± 0.44
10,882	5-Amino-1H-pyrazole-4-carbonitrile derivative	–	–	–	2.88 ± 3.07	–	53.43 ± 28.94
15,567	7-Carboxypterin	–	–	–	29.72 ± 0.41	–	83.13 ± 17.15
16,43	Nitrogen compound n.i.	–	52.65 ± 27.55	–	20.95 ± 5.91	–	–
17,236	Oleamide derivative	–	69.30 ± 10.62	–	13.51 ± 8.89	–	–
18,742	Guanosine derivative	–	–	1.93 ± 0.83	–	6.02 ± 0.02	–
ACIDS							
	Organic acids	**11.90 ± 6.64**	**1376.02 ± 367.91**	**18.20 ± 1.11**	**1037.12 ± 3.70**	**32.70 ± 12.85**	**424.20 ± 56.60**
8,73	Propanedioic acid	–	87.06 ± 28.62	–	56.97 ± 0.49	–	18.65 ± 11.64
8846	Methylmalonic acid, 2TMS derivative	–	12.72 ± 2.81	–	7.40 ± 0.06	–	–
8908	4, 6-Dioxoheptanoic acid derivative	–	–	–	–	–	3.80 ± 0.35
9,21	5-Methyl-3-isoxazolecarboxylic acid derivative	–	–	–	–	–	16.81 ± 9.23
9,74	Butanedioic acid derivative	–	–	–	–	–	56.06 ± 8.73
9753	5-Methyl-2-Furancarboxaldehyde	–	599.98 ± 167.86	–	472.18 ± 21.17	–	–
9999	2-Butenedioic acid derivative	–	–	–	10.73 ± 1.91	–	–
10,032	2-Butenedioic acid derivative	–	–	–	12.32 ± 0.79	–	21.02 ± 3.11
10,08	2-Furyl(oxo)acetic acid derivative	–	–	–	–	–	16.34 ± 2.33
10,599	Caproic acid	–	–	–	8.32 ± 0.66	–	11.29 ± 2.70
10,648	Acetopyruvic acid	–	–	–	2.34 ± 1.18	–	25.18 ± 6.62
11,164	3-Hydroxy-5-Methoxy-3-Methyl-5-Oxopentanoic acid	–	45.49 ± 14.49	–	10.72 ± 0.40	–	–
11,187	3-Methyl-2-Pentenoic acid	–	14.14 ± 4.04	–	5.10 ± 1.11	–	–
11,269	Malic acid derivative	2.88 ± 1.51	89.84 ± 8.31	3.23 ± 0.26	66.23 ± 1.94	2.82 ± 0.91	47.91 ± 6.63
11,431	Salicylic acid derivative	–	16.37 ± 1.75	–	9.60 ± 0.30	–	–
12,329	4-Hydroxybenzoic acid derivative	–	116.45 ± 117.39	–	106.08 ± 1.32	–	30.98 ± 2.33
13,22	Aconitic acid derivative	–	24.89 ± 1.81	–	14.93 ± 0.50	–	–
13,433	2,5-Dihydroxybenzoic acid derivative	–	69.40 ± 5.06	–	41.94 ± 1.59	–	26.24 ± 18.19
13,509	Azelaic acid derivative	–	16.79 ± 0.10	–	16.97 ± 0.72	–	22.27 ± 3.23
13,783	Citric acid derivative	9.02 ± 5.12	282.90 ± 23.95	11.51 ± 1.23	163.94 ± 0.59	9.39 ± 3.59	72.97 ± 10.35
14,249	D-(+)-Gluconolactone derivative	–	–	–	–	6.87 ± 2.12	–
14,336	D-(+)-Gluconolactone derivative	–	–	2.19 ± 0.20	–	6.51 ± 2.54	–
15,072	D-Gluconic acid	–	–	1.26 ± 0.18	–	7.12 ± 3.69	–
16,291	Xanthurenic acid derivative	–	–	–	31.35 ± 4.73	–	54.66 ± 0.50
	Inorganic acids	**4.39 ± 1.62**	**31.77 ± 8.68**	**10.91 ± 5.89**	**-**	**14.86 ± 5.13**	**7.82 ± 0.38**
9,4	Phosphoric acid derivative n.i.	4.39 ± 1.62	-	10.91 ± 5.89	-	14.86 ± 5.13	–
9524	Phosphoric acid derivative n.i.	–	31.77 ± 8.68	–	-	–	7.82 ± 0.38
CARBOHIDRATES							
	Sugar and derivatives	**25.81 ± 10.95**	**-**	**61.48 ± 8.51**	**-**	**102.83 ± 13.89**	**-**
12,089	Xylose derivative	-	-	0.77 ± 0.03	-	2.94 ± 1.14	-
12,878	Monosaccharide n.i.	-	-	-	-	3.13 ± 1.57	-
12,891	Monosaccharide n.i.	-	-	-	-	0.98 ± 0.20	-
13,473	Monosaccharide n.i.	3.65 ± 1.52	-	3.56 ± 1.09	-	–	–
13,651	β-D-Mannopyranoside	3.17 ± 1.47	-	3.38 ± 0.28	-	2.18 ± 0.45	–
13,738	D-Fructofuranose		-	–	-	1.82 ± 1.05	–
13,95	Monosaccharide n.i.	3.79 ± 2.33	-	4.53 ± 0.58	-	–	–
13,992	Monosaccharide n.i.	–	-	3.27 ± 0.32	-	4.23 ± 1.15	–
14,388	β-Lyxopyranose derivative	–	-	–	-	4.49 ± 0.60	–

(*continued on next page*)

Table 3 (*continued*)

R_t	Compound	F100	HF100	F75Q25	HF75Q25	Q100	HQ100
18,056	β-D-fructofuranosyl-α-D-Glucopyranoside derivative	4.95 ± 2.36	-	14.28 ± 1.3	-	29.30 ± 5.99	–
18,382	Sucrose derivative	4.46 ± 2.40	-	12.97 ± 1.54	-	25.36 ± 0.37	–
18,61	α-D-glucopyranosyl-α-D-Glucopyranoside derivative	–	-	2.02 ± 0.28	-	3.96 ± 0.74	–
18,673	β-D-fructofuranosyl-α-D-Glucopyranoside derivative	4.13 ± 1.97	-	14.32 ± 6.63	-	24.42 ± 2.11	–
19,281	α-D-Galactopyranosiduronic acid	1.66 ± 0.20	-	2.38 ± 1.82	-	-	-
	Sugar alcohols	**0.83 ± 0.14**	-	**7.30 ± 1.93**	-	**20.90 ± 4.26**	-
9416	Glycerol	0.83 ± 0.14	-	3.72 ± 1.87	–	5.58 ± 1.10	-
11,487	Erythritol derivative	-	-	-	-	0.54 ± 0.20	-
12,979	Sorbitol	-	-	-	-	1.68 ± 0.22	-
13,073	Polyalcohol n.i.	-	-	-	-	2.41 ± 0.62	-
14,537	Polyalcohol n.i.	-	-	3.59 ± 0.06	-	10.70 ± 2.12	-
TERPENES							
	Steroids	**4.17 ± 1.77**	**2653.63 ± 277.53**	**5.55 ± 1.00**	**617.10 ± 97.37**	-	-
12,304	Pregn-5-ene derivative	1.91 ± 0.83	–	2.68 ± 0.23	–	–	–
12,335	Pregn-5-ene derivative	2.26 ± 0.94	–	2.87 ± 0.77	–	–	–
16,404	Steroidal compound n.i.	–	28.31 ± 7.32	–	17.36 ± 0.29	–	–
20,492	3-5-Spirostadiene derivative	–	423.58 ± 27.15	–	93.44 ± 12.96	–	–
20,578	3-5-Spirostadiene derivative	–	200.86 ± 19.82	–	54.84 ± 4.17	–	–
21,268	Smilagenin	–	60.48 ± 9.47	–	12.58 ± 1.82	–	–
21,379	Sarsasapogenin	–	97.25 ± 16.77	–	20.44 ± 2.76	–	–
21,81	Diosgenin	–	953.93 ± 96.03	–	198.06 ± 36.93	–	–
21,894	Tigogenin	–	177.72 ± 9.73	–	33.65 ± 10.17	–	–
21,934	Yamogenin	–	396.82 ± 71.82	–	90.12 ± 12.76	–	–
22,022	Neotigogenin	–	105.64 ± 20.88	–	24.16 ± 6.25	–	–
22,946	Yucagenin	–	25.62 ± 2.82	–	9.02 ± 1.20	–	–
23,106	Gitogenin	–	134.98 ± 7.24	–	47.54 ± 6.06	–	–
23,27	Neogitogenin	–	48.45 ± 7.95	–	15.89 ± 2.00	–	–
	Triterpenoids	-	-	-	**377.74 ± 124.60**	-	**2174.47 ± 87.91**
20,72	Triterpenoid n.i.	–	–	–	–	–	10.55 ± 3.26
23,05	Triterpenoid compound n.i.	–	–	–	–	–	15.06 ± 1.02
23,672	Oleanolic acid	–	–	–	316.05 ± 97.19	–	1739.89 ± 24.87
24,56	Hederagenin	–	–	–	23.22 ± 14.71	–	225.75 ± 39.52
26,127	Serjanic acid	–	–	–	23.33 ± 4.69	–	82.76 ± 3.86
27,446	Phytolaccagenic acid	–	–	–	15.14 ± 8.01	–	100.46 ± 21.90
CARBONYL COMPOUNDS							
	Ketones	-	**65.28 ± 5.15**	-	**54.11 ± 2.27**	-	**9.62 ± 1.71**
9587	Cyclic ketone n.i.	–	65.28 ± 5.15	–	54.11 ± 2.27	–	9.62 ± 1.71

n.i.: not identified.

respect, it is interesting to remark that the final total phenolic content of the hydrolyzed fenugreek extract was almost the same than the non-hydrolyzed extract (close to 5 g/100 g of extract), but with a completely different phenolic profile from the initial one.

4. Conclusions

Thus, these findings on major bioactive compounds (saponins, sapogenins and phenolic compounds), along with the detailed additional compositions obtained by GC-MS, highlight the significant potential of co-extraction and hydrolysis treatments post-co-extraction. These methods allow modulating the content of both types of saponins or sapogenins depending on the desired predominant type, resulting in extracts that combine saponins and/or sapogenins with other compounds from the co-extracted sources, potentially combining or enhancing their biological activities. In addition, the hydrolysis process can further modify the composition, offering opportunities to tailor the co-extracts for specific applications or compounds enrichments.

Due to the limited number of studies on co-extraction process as a method to simultaneously extract compounds of interest from different plant sources, as saponin-rich sources, future research may be of interest to explore other different solvents, sample to solvent ratios and methodologies to obtain high bioactive potential co-extracts. Additionally, post-extraction transformations of extracts and co-extracts, such as hydrolysis, merit further studies as a strategy for compositional changes and potential added value of bioactive extracts. However, it is important to note that saponins, despite their wide range of bioactivities, can also exhibit toxicity. Therefore, future research should not only focus on optimizing co-extraction and hydrolysis methods to maximize bioactivity but also consider the safety of the resulting extracts.

CRediT authorship contribution statement

Emma Cantero-Bahillo: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **María de las Nieves Siles-Sánchez:** Methodology, Investigation, Formal analysis, Data curation. **Laura Jaime:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Diana Martin:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Joaquín Navarro del Hierro:** Writing – review & editing, Supervision, Conceptualization.

Funding

This study was funded by the Ministerio de Ciencia e Innovación of Spain, grant number PID 2019-110183RB-C22/MCIN/AEI/10.13039/501100011033.

Table 4
Phenolic composition (g of phenolic compound/100 g extract) of saponin-rich extracts and co-extracts from fenugreek (F) and quinoa (Q) at varying proportions, and evaluation of the effect of hydrolysis on the phenolic composition of fenugreek extract.

Compound	Q100	F50Q50	F75Q25	F90Q10	F100	HF100
Hidroquinone derivative	n.d.	n.d.	n.d.	n.d.	n.d.	0.91 ± 1.58
Hidroquinone derivative	n.d.	n.d.	n.d.	n.d.	n.d.	1.26 ± 0.39
Catechin**	0.06 ± 0.03^{a}	0.04 ± 0.01^{b}	0.02 ± 0.03^{c}	0.01 ± 0.02^{d}	nd	n.d.
Apigenin derivative	n.d.	0.07 ± 0.03^{c}	0.08 ± 0.06^{b}	0.10 ± 0.07^{a}	0.12 ± 0.10^{a}	n.d.
Vicenin II*	n.d.	0.04 ± 0.02^{c}	0.05 ± 0.03^{c}	0.05 ± 0.03^{b}	0.11 ± 0.03^{a}	n.d.
Apigenin derivative	n.d.	0.34 ± 0.05^{d}	0.38 ± 0.02^{c}	0.43 ± 0.03^{b}	0.66 ± 0.20^{a}	n.d.
Apigenin derivative	n.d.	0.13 ± 0.04^{d}	0.15 ± 0.03^{c}	0.27 ± 0.09^{b}	0.33 ± 0.01^{a}	n.d.
Apigenin derivative	n.d.	0.27 ± 0.02^{d}	0.30 ± 0.01^{c}	0.33 ± 0.08^{b}	0.47 ± 0.17^{a}	n.d.
Vainillic acid*	0.01 ± 0.00	n.d.	n.d.	n.d.	n.d.	n.d.
Homoorientin*	n.d.	0.22 ± 0.02^{d}	0.24 ± 0.07^{c}	0.39 ± 0.03^{b}	0.41 ± 0.14^{a}	0.20 ± 0.02^{b}
Apigenin derivative	n.d.	0.04 ± 0.03^{d}	0.04 ± 0.07^{c}	0.08 ± 0.07^{ab}	0.08 ± 0.02^{a}	n.d.
Orientin*	n.d.	0.15 ± 0.07^{d}	0.16 ± 0.02^{c}	0.19 ± 0.07^{b}	0.22 ± 0.09^{a}	0.57 ± 0.04^{b}
Quercetin derivative	0.57 ± 0.06^{a}	0.32 ± 0.01^{b}	0.09 ± 0.06^{c}	0.04 ± 0.07^{d}	n.d.	n.d.
Apigenin derivative	n.d.	0.12 ± 0.01^{d}	0.14 ± 0.07^{c}	0.26 ± 0.06^{b}	0.31 ± 0.01^{a}	n.d.
Typhaneoside*	0.10 ± 0.01^{b}	0.13 ± 0.12^{a}	n.d.	n.d.	n.d.	n.d.
Apigenin derivative	n.d.	0.11 ± 0.01^{c}	0.09 ± 0.03^{d}	0.12 ± 0.04^{b}	0.24 ± 0.10^{a}	n.d.
Rutin*	0.01 ± 0.01^{a}	0.01 ± 0.00^{b}	n.d.	n.d.	n.d.	n.d.
Apigenin derivative	n.d.	0.26 ± 0.05^{d}	0.29 ± 0.06^{c}	0.33 ± 0.12^{b}	0.37 ± 0.11^{a}	1.49 ± 0.22^{a}
p-coumaric acid*	0.06 ± 0.10^{a}	0.04 ± 0.03^{b}	0.02 ± 0.02^{c}	n.d.	n.d.	n.d.
Luteolin 7-*O*-glucoside*	n.d.	0.01 ± 0.00^{d}	0.02 ± 0.01^{c}	0.03 ± 0.03^{b}	0.04 ± 0.02^{a}	n.d.
Luteolin 7-*O*-glucoside*	n.d.	0.01 ± 0.02^{c}	0.01 ± 0.01^{b}	0.02 ± 0.02^{a}	0.02 ± 0.03^{a}	n.d.
Luteolin derivative	n.d.	n.d.	n.d.	n.d.	n.d.	0.17 ± 0.01
Ferulic acid*	0.02 ± 0.03^{a}	0.01 ± 0.01^{b}	n.d.	n.d.	n.d.	n.d.
Quercetin derivative	0.01 ± 0.01^{a}	0.01 ± 0.01^{b}	n.d.	n.d.	n.d.	n.d.
Salvianolic acid isomer	n.d.	0.05 ± 0.01^{d}	0.07 ± 0.04^{c}	0.09 ± 0.05^{b}	0.14 ± 0.01^{a}	n.d.
Salvianolic acid*	n.d.	0.36 ± 0.13^{d}	0.53 ± 0.03^{c}	0.63 ± 0.06^{b}	0.71 ± 0.15^{a}	n.d.
Salvianolic acid isomer	n.d.	0.39 ± 0.14^{d}	0.56 ± 0.06^{c}	0.66 ± 0.07^{b}	0.77 ± 0.11^{a}	n.d.
Luteolin	n.d.	n.d.	n.d.	n.d.	n.d.	0.05 ± 0.02
Apigenin	n.d.	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.03
Σ Phenolic compounds	**0.84 ± 0.22^{d}**	**3.13 ± 0.83^{c}**	**3.24 ± 0.70^{c}**	**4.03 ± 0.99^{b}**	**5.00 ± 1.30^{a}**	**4.76 ± 0.72**

n.d.: non-detected.
a-d Different letters in the same row indicate statistical differences among saponin-rich extracts ($p \leq 0.05$).
* Identification based on standards.

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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