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Consumers acceptance and volatile profile of wall rocket (*Diplotaxis erucoides*)

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Abstract

Wall rocket (*Diplotaxis erucoides*) is a wild edible herb traditionally consumed in the Mediterranean regions with a characteristic, pungent flavour. However, little is known about its acceptance as a potential new crop. In the present study, an hedonic test with 98 volunteers was performed in order to evaluate the potential of wall rocket as a new crop. Three products were tested corresponding to microgreens, seedlings and baby-leaves. The volatile constituents were also studied due to their probable influence on acceptance, and compared to Dijon's mustard and wasabi. The degree of acceptance was mainly related to taste and pungency. Microgreens were well accepted, whereas seedlings and baby-leaves were mainly appreciated by individuals that enjoy pungent tastes. The purchase intent was also highly related to the acceptance of taste and pungency. The volatiles profile revealed that wall rocket was rich in allyl isothiocyanate, like mustard and wasabi. This compound may be greatly responsible of the relationship between the acceptance of mustard, wasabi and wall rocket. Microgreens displayed the highest levels of isothiocyanates, although the quantity of product tested by panellists did not probably allow the appreciation of such compounds. In baby-leaves, a significant decrease in isothiocyanates GC area and relative abundances was observed. These results suggest that wall rocket microgreens would be accepted by a significant proportion of the general public since pungency is lowly perceived in the product, despite its high levels of isothiocyanates. By contrast, baby-leaves may become a crop for a cohort of consumers that enjoy pungent flavours.

Keywords

Affective test, allyl isothiocyanate, baby-leaves, GC-MS, microgreens, new crops

1. Introduction

Producers, breeders and scientists are increasing their interest in traditional wild edible plants (WEPs), which can be considered as a source of new potential crops (Shin, Fujikawa, Moe, & Uchiyama, 2018). Although underutilised, a renewed interest on WEPs has taken place during the last decades. Thus, there is an increasing number of reports that compile their traditional use, as well as studies related to nutritional traits (e.g., Guarrera & Savo, 2013, 2016; Sánchez-Mata & Tardío, 2016; Shikov, Tsitsilin, Pozharitskaya, Makarov, & Heinrich, 2017). Moreover, some WEPs have become part of local markets and are gathered and commercially exploited (Evans & Irving, 2018; Łuczaj et al., 2012). However, there are still many other underutilised vegetables that represent new opportunities for markets.

The *Brassicaceae* family, and in particular the genera *Eruca* and *Diplotaxis*, can be considered a source of potential new crops (D'Antuono, Elementi, & Neri, 2009). Among them, wall rocket (*Diplotaxis erucoides* (L.) DC. subsp. *erucoides*), a plant widespread in Mediterranean regions and Near East (Pignone & Martínez-Laborde, 2011), is an underutilised WEP with potential interest. This species is mainly consumed for the leaves, although the white flowers can be also used as decorative elements (Guijarro-Real, Rodríguez-Burruezo, Prohens, & Fita, 2018). It has been traditionally gathered in several Mediterranean countries including Spain, France or Italy (Couplan, 2015; Licata et al., 2016; Parada, Carrió, & Vallès, 2011). Although one commercial cultivar exists (cv. 'Wasabi', Shamrock Seed Company, USA), the cultivation of wall rocket is negligible.

Brassicaceae are rich in glucosinolates (GSLs), secondary metabolites that plants accumulate in different organs (Gols et al., 2018). Chemically, GSLs are sulfur- and nitrogen- containing compounds formed by a β-D-thioglucose group and a sulfonated oxime moiety, and a variable side chain derived from an α-amino acid (Ishida, Hara, Fukino, Kakizaki, & Morimitsu, 2014). The presence and predominance of individual GSLs depends on the species, although other parameters such as the developmental stage, affection of biotic/abiotic stresses and even post-harvest tasks can modify the profile as well (Bell & Wagstaff, 2017). Wall rocket accumulates sinigrin as main GSL

(Di Gioia, Avato, Serio, & Argentieri, 2018). By contrast, the related salad rocket (*Eruca sativa*) and wild rocket (*D. tenuifolia*) crops do not accumulate sinigrin but glucoraphanin, glucoerucin and glucosativin as main compounds (Di Gioia, Avato, Serio, & Argentieri, 2018).

Together with the GSLs, the cruciferous species also synthesize specific enzymes known as myrosinases When the tissue is damaged, (e.g., by chewing), the GSLs are hydrolysed by the myrosinases, realising D-glucose and an unstable aglycone that originates other compounds like isothiocyanates (ITCs) or nitriles (Bell, Oloyede, Lignou, Wagstaff, & Methven, 2018). These hydrolysis products are involved in the defensive response of plants against herbivores and pathogens (Angelino et al., 2015). On the contrary, the intake of cruciferous vegetables by humans as part of a varied diet does not release GSL-derived compounds to toxic levels (Angelino et al., 2015). Furthermore, their beneficial effects in human health have been considered for decades, and evaluated with *in vitro* and *in vivo* analyses and epidemiological studies. These compounds have shown promising results for health, including anti-inflammatory action, cardiovascular protection and reduction of cancer risk (e.g., Dinkova-Kostova & Kostov, 2012; Huang et al., 2018; Savio, da Silva, de Camargo, & Salvadori, 2014; López-Chillón et al., 2019).

Among the different classes of GSL-derived compounds, rocket crops mainly contain ITCs (Bell, Yahya, Oloyede, Methven, & Wagstaff, 2017). Different ITCs confer characteristic sulphurous, hot, or mustard-like flavours (Bell & Wagstaff, 2017), and are in general terms accepted by humans. However, the tolerance and liking perceptions for these compounds vary greatly between individuals. Aspects such as the individual GSLs profile in a specific vegetable, relative abundances and total content, but also the presence of other compounds (e.g., sugars), can affect the overall flavour of a vegetable (Bell, Methven, Signore, Oruna-Concha, & Wagstaff, 2017; Pasini, Verardo, Cerretani, Caboni, & D'Antuono, 2011), and consequently, its degree of acceptance or rejection.

In the present study, we have analysed the acceptance of wall rocket and its volatile constituents, using for this purpose two different population-varieties that are being developed as part of our breeding programme. Despite the use of wall rocket as WEP, there is little information on its organoleptic quality. As far as we know, there are only a few works describing the GSLs constituents (Bennett, Rosa, Mellon, & Kroon, 2006; D'Antuono, Elementi, & Neri, 2008; Di Gioia et al., 2018), and we have not found any report that analyse the volatiles profile. Furthermore, to our knowledge there is one unique work that evaluates the acceptance of wall rocket and other *Diplotaxis* and *Eruca* germplasm materials by twelve panellists (D'Antuono et al., 2009). These authors found a rejection of sinigrin-containing materials, such as wall rocket. However, the number of works reporting the traditional use of this wild vegetable (e.g., (Couplan, 2015; Guarrera & Savo, 2013, 2016; Licata et al., 2016; Pinela, Carvalho, & Ferreira, 2017) would indicate that it is appreciated. Here, we tested the acceptance of wall rocket by 98 potential consumers to whom it was presented as a new vegetable. Three different phenological stages (microgreens, seedlings or baby-leaves) were used to identify if consumers prefer a specific product. In addition, the volatiles profiles were evaluated for the first time, and compared to the results of the hedonic test.

2. Material and methods

2.1. Plant material and cultivation conditions

Two population-varieties of wall rocket (DER001-2 and DER006-2) were used as plant material. Materials corresponded to the second generation of wild populations collected in the region of Valencia, Spain, in April 2015, and were of high similarity in terms of ecology, morphology and phytochemical composition (Guijarro-Real, Adalid-Martínez, et al., 2019; Guijarro-Real, Prohens, et al., 2019). Population DER006-2 had, in addition, a good growing response in the earliest stages. Three different products were developed corresponding to three different developmental stages of plants. A random code of three digits was assigned to each product, as recommended for affective tests (O'Sullivan, 2017). Thus, codes P304, P355 and P398 corresponded to the microgreens, seedlings and baby-leaf stages (Fig. 1). Seeds were treated with 2.5% sodium hypochlorite followed by 100 ppm gibberellic acid solution (Guijarro-Real, Adalid-Martínez, et al., 2020). Microgreens and seedlings were obtained from accession DER006-2. Microgreens were grown in commercial Neuhaus Humin-substrat N3 substrate (Klasmann-Deilmann Gmbh, Geeste, Germany) under controlled conditions (16 h light /8 h dark, at 25ºC) for one week until the expansion of cotyledons. Treated seeds for seedlings were directly sown in the field and grown for three weeks until the expansion of the first two true leaves. Finally, DER001-2 was used for obtaining the baby-leaves. Treated seeds were directly sown in the field and grown for six weeks until reaching the pre-flowering stage. Sowings took place gradually in order to ensure the availability of the three products at the same moment. Plants in the field were placed under micro-tunnels of crop thermal blanket as protection against the low temperatures of the winter, as it is commonly used in several horticulture crops.

2.2. Consumers acceptance test

A total of 98 untrained individuals, older than 18 years old, were recruited at Universitat Politècnica de València (Valencia, Spain). Prior to the test, volunteers signed a participation consent in which the purpose of the study and the use of the provided personal data were explained. The tests filled were anonymous and treated all together in order to obtain general conclusions.

The test was performed in a single session where the three products were presented in a random order. The presentation of products was done according to Fig. 1. The quantities offered in the different stages corresponded to the expected amount that would be consumed as part of a dish preparation. Thus, for example, microgreens were presented in small amounts as they are conceived as decorative elements. Volunteers were asked to evaluate their degree of acceptance for the following sensory attributes: visual appearance, texture, taste and pungency. A Labelled Affective Magnitude (LAM) scale with 11 points ranging to the "greatest imaginable dislike" to the "greatest imaginable like" was used for scoring (Cardello & Schutz, 2004; Schutz & Cardello, 2001). Each point of the LAM scale corresponded to a numerical score ranging from -100.0 to +100.0, respectively, used for the analytical treatment of data (Fig 2). Individuals also declared their purchase intent for the different products using a 1-5 scale (1= absolutely no; 5= absolutely yes). After the test, participants filled a personal questionnaire adapted from Bell, Methven, & Wagstaff

(2017). In this test, their preferences for ruccola, Dijon's mustard and wasabi paste (1 = like; 2 = do not like; 3 = never tried) were also asked (Table 1).

2.3. Extraction and analysis of volatile organic compounds (VOCs)

In the analysis of VOCs, commercial Dijon's mustard (from now on, mustard; Reine de Dijon SAS, France) and wasabi paste (from now on, wasabi; Kaneku Corp., Japan) were included as references. Extraction was performed by means of the headspace-solid phase microextraction (HS-SPME) technique, and the volatile compounds obtained were analysed by gas chromatography–mass spectrometry (GC–MS). For this purpose, subsamples of 1.5 g of fresh material were used. Samples of wall rocket were finely chopped with a knife in slices that were approximately 0.5 cm long, whereas 1.5 g of homogenised paste were used for mustard and wasabi. The processed samples were placed into 20 mL sealed headspace vials within 1 min. The extraction and analysis conditions were as described in Guijarro-Real, Rodríguez-Burruezo, Prohens, Raigón, & Fita (2019). Samples were pre-incubated at 40ºC for 30 min, then VOCs were adsorbed for 40 min on a fibre (50/30 µm DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA). A thermal desorption step was carried out at 250ºC for 30 s using the splitless mode. Four replicates were analysed.

The analysis was performed using a 6890 N Network GC System with autosampler coupled to a 5973 Inert Mass Selective Detector (Agilent Technologies; Santa Clara, CA, USA) and equipped with a HP-5MS J&W silica capillary column (5% phenyl-95% methylpolysiloxane, 30 m x 0.25 mm i.d., 0.25 µm thickness film; Agilent Technologies). As carrier gas, helium was used at a flow of 1 mL min^{-1}. The temperature gradient of the column started at 100ºC and raised to 250ºC at a rate of 5ºC min^{-1}, then maintained for 10 min. For the detection by the mass spectrometer, the electron impact mode (EI 70 eV ionization energy, source temperature at 225ºC) was used, with acquisition performed in the scanning mode (mass range *m/z* 35–350 amu).

The MSD ChemStation D.02.00.275 (Agilent Technologies) was used for processing chromatograms and spectra. A tentative identification was obtained by comparison of the mass spectra with the NIST 2005 Mass Spectral Library, and also comparing the retention times and mass spectra with our customised library. Compounds scoring > 80% match for mass spectra were considered (Bell, Spadafora, Müller, Wagstaff & Rogers, 2016). A semi-quantification was obtained based on the integration of peak areas by the total ion current chromatogram (Guijarro-Real, Rodríguez-Burruezo, et al., 2019; Moreno, Fita, González-Mas, & Rodríguez-Burruezo, 2012), and the relative abundance or percentage of each compound was also estimated as the ratio between its peak area and the added area of the total compounds identified.

2.4. Statistical analysis

The influence of the categorical data in the affective test was studied using a χ^2 test for heterogeneity (*P* = 0.05). Questionnaires were clustered for a deep analysis of results (Bell, Methven, & Wagstaff, 2017). Two different clustering options were established: according to the volunteers' preferences for a) mustard, or b) wasabi. These groups were the most homogeneous in number of individuals and were also considered due to the possible association that may be established between acceptance of wall rocket and both mustard and/or wasabi. Three groups were established in each case corresponding to the answers provided by the individuals (1 = like, 2 = do not like, 3 = never tried). Questionnaires that did not answer to these questions were not included in the analysis. Mean values and standard errors (SE) for visual appearance, texture, taste and pungency, and purchase intent were calculated: 1) considering all questionnaires as a whole (*n* = 98); and 2) after clustering. Data were analysed with the Kruskal-Wallis test, and the signification of differences were calculated with the Bonferroni procedure (*P* = 0.05) (Dinnella, Torri, Caporale, & Monteleone, 2014). The purchase intent was transformed from the 5-point scale to a 3-point scale (1-2 = low; 3 = medium; and 4-5 = high). The correlations between attributes and purchase intent were studied and Spearman's rank coefficients of correlation (ρ) calculated. All statistical analyses were performed with the Statgraphics Centurion XVII software (Statpoint Technologies, Inc., Warrington, VA, USA), with the exception of the test for heterogeneity that were calculated on Excel sheets.

For the analysis of volatiles profiles, mean values of GC peak areas and SE were obtained (n = 4). Relative abundances of each compound were also calculated against the total identified in each sample and expressed as percentage (Guijarro-Real, Rodríguez-Burruezo, et al., 2019). Absolute area GC-peak area data were $\log_2$-transformed for normalization (Sdiri, Rambla, Besada, Granell, & Salvador, 2017), and then subjected to a one-way factorial analysis of variance (ANOVA) to compare each individual volatile among materials. Signification was evaluated using the Student-Newman-Keuls test (P = 0.05). An illustrative comparison of profiles was performed by means of a Hierarchical Cluster Analysis (HCA), using the ClustVis tool (Metsalu & Vilo, 2015). The distance metrics was based on the Pearson correlation and rows were clustered according to the average linkage (López-Gresa et al., 2017).

3. Results

3.1. Profile of panellists

The personal profile of the ninety-eight volunteers is given in Table 1. Five participants (5.1%) did not answer the personal questionnaire, percentage that rose to 13.3% when the age was asked. Results showed a similar participation of males and females (46.9% and 48.0%, respectively), mainly European coming from Mediterranean regions (76.5%). The range age 18–27 years old was the most abundant (40.8%), while the percentage of people older than 48 years only represented 10.2% of the panel (Table 1).

Regarding their preferences, a total of 82.7%, 46.9% and 37.8% of participants indicated their positive preferences for ruccola, mustard and wasabi, respectively (Table 1). Finally, the percentage of participants who had never tried mustard was higher than for those ones who had never tried wasabi (32.7% and 18.4%, respectively).

3.2. General perceptions of the three products

Positive scores were on average obtained for the four attributes across the three products (Fig. 3a). From all attributes, pungency displayed the lowest values. The purchase intent was on average > 3 in a 1-5 scale (Fig. 3b). No significant differences were determined among products either for attributes or the purchase intent. As exception, the preference for the visual appearance of seedlings was lower than for microgreens and baby-leaves (Fig. 3a).

However, data displayed very wide ranges along the LAM scale as well as for the purchase intent (Fig. 3). Thus, a deep analysis was performed based on clustering the questionnaires as explained in the Material and Methods section. The test for heterogeneity revealed remarkable significant differences between groups when the individuals were grouped by their preferences (Table S1). From the three options, the preferences for mustard and for wasabi provided the most homogeneous groups that were in all cases below 50% (Table 1). There, two clustering options were selected for the analyses, and corresponded to the preferences for mustard (A) and/or wasabi (B).

3.3 Affective test by cohorts

Mean scores for the two clustering options are summarised in Table 2. Overall, both the visual appearance and the texture had positive scores, with no significant differences between groups or products. These values corresponded to a moderate-high acceptance according to the LAM scale. As exception, the visual appearance score of seedlings was lower than for microgreens and baby-leaves in group A.3 ("never tried mustard"), as it was previously detected in the general perceptions analysis (Table 2, Fig. 3).

The taste and pungency of microgreens was also appreciated by all groups, with a slight-moderate acceptance according to the LAM scale. No significant differences among clusters were determined for these traits (Table 2). Nevertheless, some individuals indicated a lack of pungency in microgreens, attribute that was perceived as positive for individuals who do not like mustard or wasabi, but negative for those ones who like them. On the contrary, the acceptance of both seedlings and baby-leaves was significantly affected by individual preferences. Thus, groups A.1 and B.1 (like mustard or wasabi, respectively) displayed the highest scores (> 33), indicating a moderate-high preference (Table 2). By contrast, groups A.2 and B.2 (do not like mustard or wasabi, respectively), and groups A.3 and B.3 (have never tried mustard or wasabi, respectively) had on average low scores or negative scores. The pungency attribute had the lowest scores, up to -

33.6 (seedlings) (Table 2). In fact, we found comments in the questionnaires indicating they did not like these products because they do not like pungent flavours.

On the other hand, significant differences were established between products in the clustering option A (Table 2). For group A.1, the mean scores for seedlings and baby-leaves were positive and similar (> 37), and higher than the mean score for microgreens. On the contrary, the group A.2 had a positive, high mean score for microgreens but negative for seedlings and baby-leaves. Finally, the cluster A.3 had the highest value for the taste of microgreens, significantly different from the score for seedlings. Interestingly, no significant differences among products were found for the clustering option B (Table 2).

3.3.1 Relationship between affective scores and purchase intent

The purchase intent was positively correlated with all traits at a high level (Table 3). The highest coefficients of correlation were determined for the acceptance of taste and pungency, ranging between 0.651 (microgreens) and 0.685 (seedlings) for taste, and between 0.442 (microgreens) and 0.638 (seedlings) for pungency. Among traits, the highest coefficient was indeed determined for the acceptance of taste and pungency, with a general value of 0.5254 (data not shown).

3.4. Volatile constituents

Important differences were found in the volatiles profiles of microgreens, seedlings and baby-leaves (Table 4). The profile of the microgreens was mainly composed by ITCs, with ten compounds accounting for 98.2% of total area. Allyl ITC was the most representative compound (95.7%). In a similar way, the profile of the seedlings was also mainly represented by ITCs followed by esters (78.2% and 17.4% of the total area, respectively). Seven compounds were identified in each chemical group (Table 4). Allyl ITC was also the main compound in this profile, but it was lower in absolute level and relative abundance in comparison with microgreens. Other compounds of relevance in the seedlings were *cis*-3-hexenyl butyrate (6.8%), with similar relative abundance to P398 but higher GC area; and *cis*-3-hexenyl isovalerate (5.4%), similar to the baby-leaves in GC area. Surprisingly, the baby-leaves had a very poor profile in quantitative terms (Table 4). This product was rich in esters, accounting for 94.1% of total area. C*is*-3-hexenyl isovalerate (62.5%) and *cis*-3-hexenyl valerate (13.3%) were the most representative compounds in this fraction. The isothiocyanates fraction was only determined by allyl ITC, which had a relative abundance of 4.9% of the total.

These differences were reflected in the HCA (Fig. 4). Samples of mustard and wasabi were also included in this analysis. Compounds were grouped in two main clusters, C1 and C2. Cluster C1 included the esters and alcohols, which were only detected in wall rocket. Within this, subcluster C1.1 included four esters that were only detected in the seedlings and some replicates of the baby-leaves. On the other hand, cluster C2 was mainly composed by GSLs breakdown products (Fig. 4). This cluster mainly related the volatiles profiles of mustard and wasabi with wall rocket microgreens, and to a lesser extent, the seedlings. Allyl ITC was also the main compound detected in mustard and wasabi. From all materials, wasabi had the highest levels of allyl ITC. Phenylethyl ITC was also of in high levels in wasabi (31.3%), more than 200-fold times greater than in the microgreens and almost 500-fold times higher than the seedlings. In addition, three compounds in this cluster were determined as unique for certain materials: 4-methyl-thiobutanenitrile specific for mustard, 2-methoxy-3-(1-methylpropyl)-pyrazine for wasabi, and *β*-ionone for the microgreens. Butyl ITC was the only compound detected in both mustard and wasabi but not found in wall rocket.

4. Discussion

This work analyses for the first time the acceptance of wall rocket as a new crop in a large number of potential consumers, and it is also the first report describing the volatiles profile of the species. The most representative profile of volunteers participating in the hedonic test corresponded to young to middle age Mediterranean Europeans, with no gender bias. Thus, the information provided in this study could be of great relevance for the potential commercialization of wall rocket in markets of Mediterranean countries. By contrast, new studies may be needed for other markets.

Our hedonic test results differ greatly from the work of D’Antuono et al. (2009). Such difference may be in part due to the study design. The current work was addressed to evaluate the acceptance of wall rocket as a new vegetable, while the study of D’Antuono et al. (2009) compared wall rocket with ruccola and other related germplasm. However, the profile of GSLs in wall rocket and ruccola is different (Di Gioia et al., 2018), and consequently, the flavour would be different (Bell et al., 2018). Therefore, the presentation of both species together may negatively affect the perception of wall rocket, since a similar taste and aroma to ruccola could be expected.

Clustering the participants of a hedonic test can be useful for the analysis of data that show broad ranges (Bell, Methven, & Wagstaff, 2017; Dinnella et al., 2014). The visual appearance was mainly accepted by all groups. The visual appearance of the product is probably the most important one, and in vegetables is usually related to freshness (Dinnella et al., 2014). The current study was developed using fresh material that was harvested the same day of analysis. However, it can be negatively affected by the post-harvest manipulation and during shelf-life (Shewfelt, Prussia, & Sparks, 2014). Therefore, the future commercialization of wall rocket should consider previous studies aimed to conserve the fresh appearance during post-harvest conditions. Moreover, our results showed a reduced acceptance of seedlings compared to the other products. This lower score may correspond to the low resemblance of this phenological stage to other leafy vegetables. In fact, Bell, Methven, & Wagstaff (2017) suggested that the acceptance of new vegetables tends to increase when they are somehow similar to other commercial products. Therefore, this study suggests that P355 would be the less desirable stage for a commercial purpose.

On the other hand, microgreens have gained in popularity during the last decades and are broadly used in restaurants as decorating components. Within the *Brassicaceae* family, species like radish or mustard are commercially grown as microgreens (Xiao, Lester, Luo, & Wang, 2012), and our results suggest that wall rocket may become part of this market. The fact that those individuals who reject mustard and wasabi appreciated the microgreens stage indicates that consumers may not find an associated flavour between wall rocket microgreens and mustard/wasabi, despite the fact that their ITCs profile was highly similar. A possible explanation may be related to the low amount of microgreens that was tested by panelists, which was offered according to the expected commercial use of this product. On the contrary, the taste and pungency of the seedlings and baby-leaves were highly responsible for the rejection and the low purchase intent that some cohorts exposed. Taste and pungency are two attributes with positive correlation in *Brassicaceae* (D'Antuono et al., 2009). These traits are highly related to the presence of GSLs (Bell et al., 2018), and usually influence the acceptance of crops in this family (Wieczorek, Walczak, Skrzypczak-Zielińska, & Jeleń, 2017). However, our results suggest that baby-leaves may be also considered as a market opportunity addressed to the cohort of consumers that enjoy "mustard-like" flavours.

Regarding the volatiles profiles, allyl ITC was the main ITC in wall rocket, mustard and wasabi. Therefore, this compound might account for the relationship between acceptance of wasabi/mustard and wall rocket. According to our results, allyl ITC has been previously described in the materials of reference (Bell et al., 2018), as well as in other crops such as horseradish or Brussels sprouts (Agneta et al., 2014; Ishida et al., 2014). In a similar way, it was expected that wall rocket materials were rich in allyl ITC. Wall rocket has been previously described as rich in sinigrin (D'Antuono et al., 2008, 2009; Di Gioia et al., 2018) and this GSL is the precursor of allyl ITC (Cavaiuolo & Ferrante, 2014), explaining thereby its high abundance in wall rocket's VOCs profile.

Another compound detected that would be of great interest was diallyl disulphide. This VOC represented less than 0.1% of the total area in the microgreens, and was determined as traces in seedlings and baby-leaves. Diallyl disulphide has been described as one of the main sulphur compounds of fresh garlic (Molina-Calle, Priego-Capote, & Luque de Castro, 2017) and, like other sulphur compounds, it provides pungent and intensive garlic notes (Ma et al., 2011). In addition, a very low odour threshold has been described for sulphur compounds (Nagata, 2003). Thus, its presence in the materials, even as traces, could explain the "garlic notes" that some participants described in the present study and also related in the work of D'Antuono et al. (2009).

Finally, the volatiles profile of baby-leaves was unexpected due to the lack of ITCs and the high relative abundance in esters. A previous study showed that the both varieties used in the present work accumulated similar contents in sinigrin for the same phenological stage (Guijarro-Real, Adalid-Martínez, et al., 2019). However, plants used for the baby-leaf product in this study were exposed to colder growing conditions due to its early sowing. The different environmental conditions in which they were grown, as well as the difference in the phenological stage, may have affected the biosynthesis and accumulation of GSLs. Unlike in our case, the trend of plants is to accumulate GSLs under stress (Bonasia, Lazzizera, Elia, & Conversa, 2017). Thus, new experiments in different growing conditions and locations should be repeated in order to better understand the variations in taste that wall rocket can suffer, which is primordial for crop quality homogenization.

5. Conclusions

To our knowledge, this is the first report in evaluating the volatiles profile of wall rocket. The species was rich in ITCs, although baby-leaves displayed very low levels. Allyl ITC was the main ITC identified in wall rocket, seedlings and baby-leaves, which presumably influence their flavour similarities.

Results of the hedonic tests indicated that the acceptance was mainly related to the preferences for taste and pungency. Microgreens, tested as decorative element in small amounts, were well accepted by all panelists. By contrast, seedlings and baby-leaves were also accepted by consumers that enjoy pungent flavours, although the poorer visual appearance of seedlings made them less interesting. Thus, the study suggests that both microgreens and baby-leaves of wall rocket may be considered as good market opportunities, the former for the general public, the later for a more specific cohort of consumers.

Declarations of interest

None.

Author contributions

C. Guijarro-Real developed the methodology, collected data, performed the statistical analysis, and wrote the manuscript. J. Prohens designed the experiment, performed the statistical analysis, and wrote/edited the manuscript. A. Rodríguez-Burruezo designed the experiment, assisted in the methodology, and edited the manuscript. A. Fita designed the experiment, developed the methodology, assisted in statistical analysis and wrote/edited the manuscript.

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Table 1. Personal questionnaire asked to the volunteers. Data are expressed as percentage against total participants (n = 98).

Individuals' profile (%)					
Gender		**Age**		**Origin**	
Female	48.0	18-27	40.8	Mediterranean-European	76.5
Male	46.9	28-37	14.3	South American	10.2
NA[a]	5.1	38-47	21.4	Asiatic	4.1
		48-57	7.1	Nordic/Central European	4.1
		58-76	3.1	NA	5.1
		NA	13.3		
Preference for... (%)					
Ruccola		**Dijon's mustard**		**Wasabi paste**	
Yes	82.7	Yes	46.9	Yes	37.8
No	9.2	No	15.3	No	38.8
Never tried	3.1	Never tried	32.7	Never tried	18.4
NA	5.1	NA	5.1	NA	5.1

[a] NA= not answered

Table 2. Mean scores for visual appearance, texture, taste and pungency attributes after clustering for Dijon's mustard preference (A) or wasabi paste preference (B). Groups are established according to the acceptance/rejection to mustard or wasabi: 1 = like, 2 = do not like, 3 = never tried. *N* indicates the number of panelists included in each cluster.

		Visual appearance			**Texture**			**Taste**			**Pungency**		
Group	***N***	**Microgreens**	**Seedlings**	**Baby-leaves**	**Microgreens**	**Seedlings**	**Baby-leaves**	**Microgreens**	**Seedlings**	**Baby-leaves**	**Microgreens**	**Seedlings**	**Baby-leaves**
Clustering option A. Preference for Dijon's mustard													
1	*46*	45.8$^{-/-}$	31.5$^{-/-}$	46.8$^{-/-}$	31.5$^{-/-}$	38.8$^{-/-}$	44.7$^{-/-}$	18.2$^{-/A}$	39.9$^{b/B}$	37.7$^{b/B}$	10.7$^{-/A}$	37.2$^{b/B}$	41.2$^{b/B}$
2	*15*	40.2$^{-/-}$	19.4$^{-/-}$	28.9$^{-/-}$	28.4$^{-/-}$	18.4$^{-/-}$	27.4$^{-/-}$	29.5$^{-/-}$	-4.7$^{a/-}$	0.3$^{a/-}$	36.2$^{-/B}$	-33.6$^{a/A}$	-16.7$^{a/A}$
3	*32*	50.6$^{-/B}$	23.9$^{-/A}$	38.2$^{-/AB}$	41.6$^{-/-}$	29.7$^{-/-}$	34.0$^{-/-}$	33.9$^{-/B}$	2.2$^{a/A}$	16.0$^{ab/AB}$	15.3$^{-/-}$	-9.5$^{a/-}$	-6.1$^{a/-}$
Clustering option B. Preference for wasabi paste													
1	*37*	51.8$^{-/-}$	32.1$^{-/-}$	43.7$^{-/-}$	29.4$^{-/-}$	36.4$^{-/-}$	40.1$^{-/-}$	21.6$^{-/-}$	37.7$^{b/-}$	35.7$^{-/-}$	22.4$^{-/-}$	33.8$^{b/-}$	39.9$^{b/-}$
2	*38*	44.8$^{-/-}$	24.6$^{-/-}$	37.2$^{-/-}$	41.4$^{-/-}$	34.9$^{-/-}$	40.7$^{-/-}$	25.7$^{-/-}$	8.3$^{a/-}$	15.6$^{-/-}$	13.5$^{-/-}$	-11.2$^{a/-}$	2.7$^{a/-}$
3	*18*	40.0$^{-/-}$	21.1$^{-/-}$	43.1$^{-/-}$	30.5$^{-/-}$	18.6$^{-/-}$	29.3$^{-/-}$	33.0$^{-/-}$	7.2$^{a/-}$	18.7$^{-/-}$	11.6$^{-/-}$	4.4$^{ab/-}$	-7.2$^{a/-}$

Different letters within columns for each clustering option (lower case), or within rows for each attribute (capital letters) indicate significant differences according to the Bonferroni procedure (P = 0.05).

Table 3. Mean scores for each attribute after grouping questionnaires by the expressed purchase intent (low, medium or high). Spearman's rank coefficient of correlation (ρ) between each attribute and purchase intent is also indicated.

	Purchase intent			
	Low	Medium	High	ρ
Visual appearance				
Microgreens	35.3[a]	37.0[a]	64.2[b]	0.335[**]
Seedlings	16.7[a]	27.2[a]	35.6[a]	0.193[***]
Baby-leaves	25.1[a]	38.8[a]	54.5[b]	0.367[***]
Texture				
Microgreens	18.8[a]	27.3[a]	51.2[b]	0.341[***]
Seedlings	14.9[a]	33.8[ab]	47.5[b]	0.332[***]
Baby-leaves	24.9[a]	35.3[a]	49.8[b]	0.336[**]
Taste				
Microgreens	-4.2[a]	19.9[b]	52.9[c]	0.651[***]
Seedlings	-22.8[a]	20.9[b]	56.1[c]	0.685[***]
Baby-leaves	-22.2[a]	27.4[b]	55.0[c]	0.666[***]
Pungency				
Microgreens	-7.2[a]	11.4[a]	42.1[b]	0.442[***]
Seedlings	-37.5[a]	8.3[b]	53.4[c]	0.638[***]
Baby-leaves	-25.6[a]	21.9[b]	40.1[b]	0.513[***]

Different letters within rows indicate significant differences according to the Bonferroni procedure (P = 0.05). [**] and [***] indicate significance at P = 0.01 and 0.001, respectively.

Table 4. Average GC peak areas (x10^6; ±SE), relative abundance (%, between brackets) and retention index (RI) for individual volatile organic compounds (VOC) identified in P304, P355 and P398 (*n* = 4).

VOC	**RI**	**Microgreens**	**Seedlings**	**Baby-leaves**
Alcohols				
cis-3-hexen-1-ol	868	12.6±1.2^b	27.0±5.8^b	0.5±0.2^a
		(1.1)	(4.4)	(0.7)
Aldehydes				
benzeneacetaldehyde	1081	0.2±0.0^a	-‡	-
		(0.0)		
decanal	1204	0.15±0.0^a	-	0.2±0.1^a
		(0.0)		(0.2)
Esters				
cis-3-hexenyl acetate	992	-	22.5±2.6^b	6.4±2.2^a
			(3.6)	(10.3)
cis-3-hexenyl propionate	1091	-	7.5±1.1^a	0.5±0.4^a
			(1.2)	(0.7)
hexyl butyrate	1183	0.1±0.0^a	0.7±0.4^a	-
		(0.0)	(0.1)	
cis-3-hexenyl butyrate	1191	5.6±2.1^a	42.1±18.3^b	4.4±3.3^a
		(0.5)	(6.8)	(7.0)
cis-3-hexenyl isovalerate	1226	0.6±0.1^a	33.2±7.7^a	39.0±35.2^a
		(0.1)	(5.4)	(62.5)
cis-3-hexenyl valerate	1290	-	8.3±1.5^a	11.0±2.7^a
			(1.3)	(13.3)
3-hexenyl benzoate	1565	-	0.4±0.1^a	0.2±0.1^a

			(0.1)	(0.4)
Ketones				
β-ionone	1457	1.3±0.3[a] (0.1)	tr[§]	-
Isothiocyanates (ITCs)				
allyl ITC	846	1140.7±76.2[c] (95.7)	462.7±47.9[b] (75.0)	3.0±0.7[a] (4.9)
isobutyl ITC	926	0.7±0.1[a] (0.1)	-	-
3-butenyl ITC	951	11.0±1.9[b] (0.9)	4.3±0.7[a] (0.7)	-
3-methylbutyl ITC	1041	6.6±0.8[a] (0.6)	5.3±0.9[a] (0.9)	-
pentyl ITC	1077	2.3±0.1[a] (0.2)	tr	-
4-methylpentyl ITC	1136	0.1±0.0[a] (0.0)	0.0±0.0[a] (0.0)	-
hexyl ITC	1185	0.6±0.1[a] (0.0)	-	-
3-methylthipropyl ITC	1287	1.0±0.4[a] (0.1)	0.5±0.3[a] (0.1)	-
benzyl ITC	1318	3.1±0.9[a] (0.3)	0.1±0.0[a] (0.0)	-
phenylethyl ITC	1429	4.3±1.4[a] (0.4)	2.0±1.2[a] (0.3)	-
Monoterpenes				

limonene	1018	-	0.5±0.1[a] (0.1)	-
Nitriles				
benzenepropanenitrile	1238	0.2±0.1[a] (0.0)	0.3±0.2[a] (0.0)	-
Sulphur compounds				
diallyl disulphide	1099	0.2±0.1[a] (0.0)	tr	tr
Total isothiocyanates		1170.5±81.4[c] (98.2)	482.4±51.6[b] (78.2)	3.5±1.1[a] (4.9)
Total esters		6.4±2.3[a] (0.5)	107.1±19.6[b] (17.4)	58.3±45.6[ab] (94.2)
Total		1191.5±78.3[b]	617.3±76.7[b]	62.3±46.9[a]

Different letters within rows indicate significant differences for log_2-transformed data according to the Student-Newman-Keuls test ($P = 0.05$).

‡-: not detected. §tr: traces

Fig. 1. Presentation of the products developed for the current study. A) Microgreens, identified in the affective test as product P304. B) Seedlings, identified as product P355. C) Baby-leaves, identified as product P398.

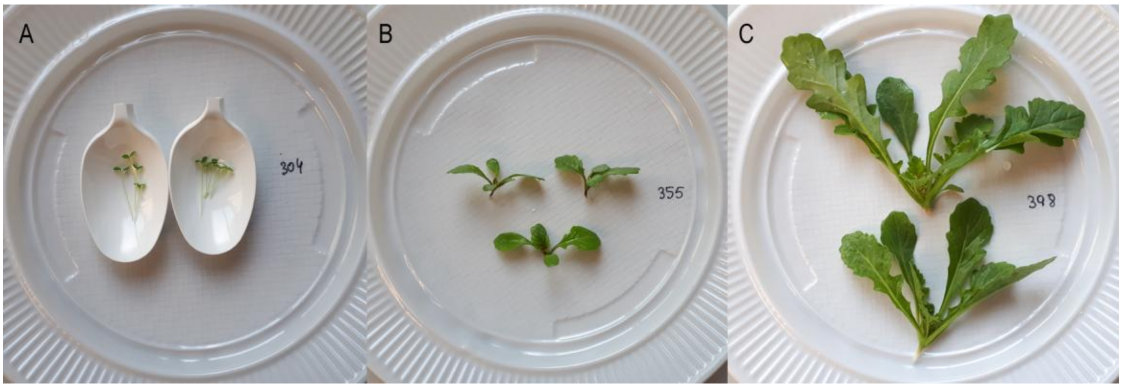

Fig. 2. Labelled Affective Magnitude (LAM) scale used in the present study. The scale includes nine points of like appreciations, ranging from the "greatest imaginable dislike" to the "greatest imaginable like". Scores corresponding to each affective point and used for the statistical analysis of data are provided in parentheses. Cardello & Schutz (2004).

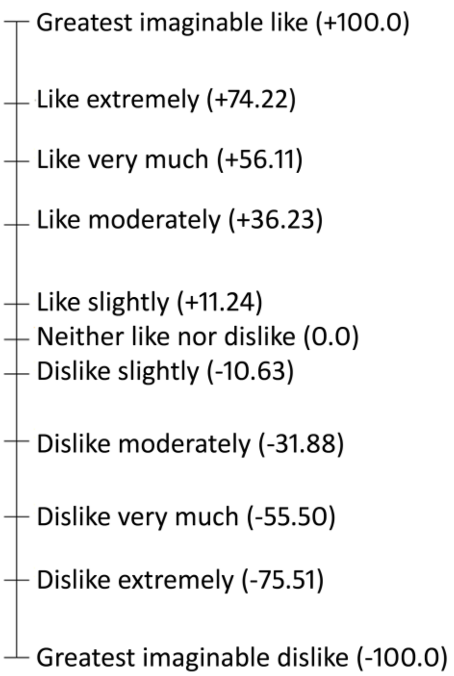

Fig. 3. Box plot charts for the traits evaluated in microgreens, seedlings and baby-leaves (n = 98). A) Box plot chart for visual appearance, texture, taste, and pungency, with a scale ranging from -100 to +100, and being -100 "the greatest imaginable dislike" and +100 "the greatest imaginable like" according to the LAM scale. B) Box plot chart for the purchase intent, with a scale ranging between 1 (absolutely no) and 5 (absolutely yes). The mean (+), median (–) and outliers values are represented.

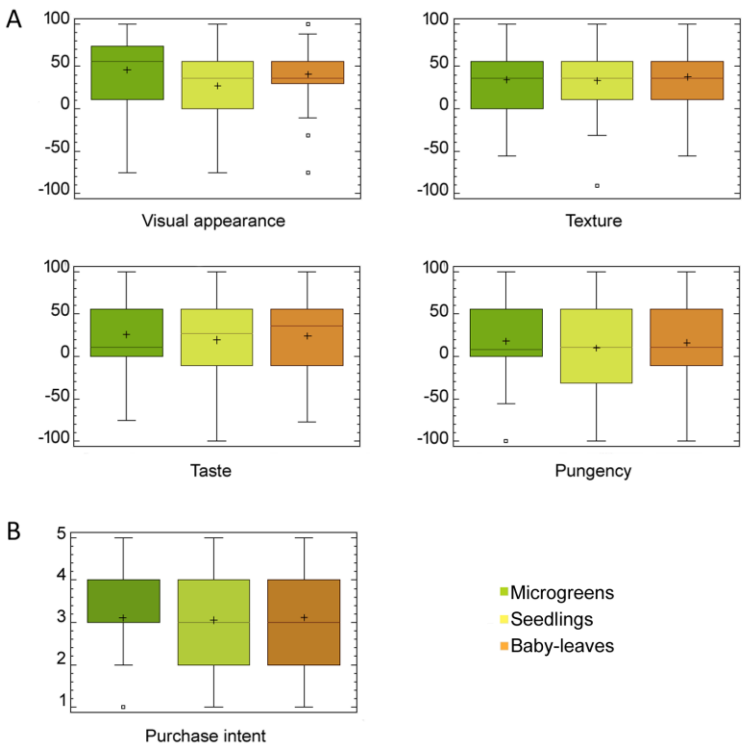

Fig. 4. Hierarchical cluster analysis of the identified volatile compounds in the three samples of wall rocket: microgreens, seedlings and baby-leaves, Dijon's mustard and wasabi paste (n = 4).