



Characterisation of mucilages extracted from seven Italian cultivars of flax



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ABSTRACT

The chemical composition, physicochemical, functional and sensory properties of mucilages, extracted from seven Italian flax cultivars, were evaluated. All samples were composed of neutral and acidic sugars, with a low protein content. From the NMR data, a rhamnogalacturonan backbone could be inferred as a common structural feature for all the mucilages, with some variations depending on the cultivar. All the suspensions showed a poor stability, which was consistent with a low zeta potential absolute value. The viscosity seemed to be positively correlated with the neutral sugars and negatively with the amount of proteins. Functional properties were dependent on the cultivar. The sensory analysis showed that most mucilages are tasteless. All these outcomes could support the use of flaxseed mucilages for industrial applications. In particular, Solal and Festival cultivars could be useful as thickeners, due to their high viscosity, while Natural, Valoal and Kaolin as emulsifiers for their good surface-active properties.

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1. Introduction

Mucilage is a common constituent of plants, which can be extracted from the seeds or soft stems, e.g. okra (from *Hibiscus esculentus*), psyllium (from *Plantago* species), yellow mustard (from *Sinapis alba*) and flaxseed (from *Linum usitatissimum*) (Izydorczyk, Cui, & Wang, 2005). The flaxseed coat, together with the endosperm, forms six layers. Mucilage comes from the secondary wall material in the outermost layer (Daun, Barthet, Chornick, & Duguid, 2003). It is easily extracted from the seed coat by soaking in water. When hydrated, the mucilage cells swell, and their content exude on the surface of the seeds. Mucilage makes up approximately 8% of the total seed weight. Several reports showed that extraction yield, protein content and physicochemical properties of the resulting mucilage depend on both the procedure and the variety of the raw material (Barbary, Al-Sohaimy, El-Saadani, & Zeitoun, 2009; Fedeniuk & Biliaderis, 1994).

Flax mucilage contains between 50–80% carbohydrates and 4–20% proteins and ash. The major constituent of flax mucilage consists of two polysaccharide components, neutral and acidic. The neutral fraction contains L-arabinose, D-xylose and D-galactose in a mole ratio of 3.5:6.2:1 and the acidic fraction contains L-rhamnose, L-fucose, L-galactose, and D-galacturonic acid in a mole ratio of 2.6:1.4:1.7. The neutral polymer is composed of an arabinoxylan with a (1/4)β-D-xylan backbone, to which arabinose and galactose side chains are attached at positions 2 and/or 3. The acidic polymer has a backbone of (1/2)-linked α-L-rhamnopyranosyl and (1/4)-linked D-galactopyranosyluronic acid residues, with side chains of fucose and galactose (Oomah, Kenaschuk, Cui, & Mazza, 1995).

The utilisation of mucilage gums depends on their unique functional properties, such as viscosity, emulsifying and foaming properties, gelation and water binding as well as on their bio-active role in the prevention and/or treatment of certain diseases. For example with its lubrication and moisturizing characteristics, flaxseed gum has been used as a bulk laxative and as a suspending agent in barium sulphate suspensions for x-rays (Izydorczyk et al., 2005). Flax mucilage is soluble in cold water and exhibits Newtonian flow behaviour at low concentrations and shear thinning flow at high polymer concentrations. It is a low to medium viscosity

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gum, and the viscosity is markedly influenced by salt concentration and pH. The maximum solution viscosity was obtained in the neutral pH zone (pH 6–8) and a zero salt concentration (Fedeniuk & Biliaderis, 1994; Mazza & Biliaderis, 1989). Cui, Mazza, and Biliaderis (1994) reported that the neutral fraction of the flax mucilage is composed mainly of arabinoxylans with a large molecular size, and exhibits shear thinning behaviour at higher concentrations, whereas the acidic fraction contained a pectic-like material with much smaller molecular sizes, and exhibited Newtonian flow behaviour. Flax mucilage also exhibits surface activity and the ability to stabilize oil/water emulsions and foams. It has been used in emulsion preparation in order to enhance stability. It stabilizes the emulsions by increasing the viscosity and decreasing the interfacial tension (Khalloufi, Corredig, Goff, & Alexander, 2009). In addition to guar gum, locust bean gum, or arabic gum, which are the most common commercial gum products, the flaxseed mucilage can be used as a thickener, stabilizer, and water-holding agent (Mazza & Biliaderis, 1989). Mucilage from flaxseed has been traditionally used as an egg white substitute in bakery and ice cream products. Recently, flaxseed mucilage has also been used in cosmetics, to give a smooth feeling to creams and lotions and it is used as a saliva substitute because it possesses lubricating and moisture-retaining characteristics resembling those of natural saliva (Izydorczyk et al., 2005).

However, flax mucilage has not been widely utilised due to limited information about the chemical composition, the functional properties and the sensory profile. Knowledge of these characteristics could significantly broaden the range of flax mucilage applications and commercial value. Therefore, the aim of this research was to extract flax mucilages from seven Italian cultivars to study their chemical compositions (i.e. neutral and acidic sugar ratio and protein content), physicochemical properties (i.e. zeta potential, conductivity, particle size and viscosity) and functional properties (solubility, swelling power, foaming and emulsifying capacity). Furthermore, all samples were characterised by NMR experiments in order to establish some details of their structure and to correlate the chemical, physicochemical and functional properties with the structural characteristics of their constituents. Moreover, a sensory profile of all mucilage samples was carried out. This study provides useful information regarding flaxseed mucilage chemical features for their application as additives in food, cosmetic or pharmaceutical industry processes. Therefore, the cultivar-dependent properties were investigated to identify the best flax variety for a specific industrial application.

2. Materials and methods

2.1. Materials

Seven Italian flax seed cultivars (*Linum usitatissimum*), namely Valoal, Linoal, Merlin, Kaolin, Natural, Festival and Solal, were obtained from the Institute of Agricultural of Biology and Biotechnology (IBBA), (Milan, Italy) during 2011/2012. A voucher specimen was stored and preserved in our laboratory, at the Department of Chemistry and Industrial Chemistry at the University of Genoa. Seeds were stored in an air-tight container at 4 °C in the dark until use.

2.2. Reagents

Anthrone, iron (III) chloride, orcinol, D-galacturonic acid, resorcinol and D-xylose were purchased from Sigma (Milan, Italy). Potassium sulfamate, sodium tetraborate and m-hydroxydiphenyl were obtained from Alfa Aesar (Karlsruhe, Germany).

2.3. Mucilage powder preparation

Mucilage in the seed coat of flaxseed was extracted following a method modified from Wang, Li, Wang, Li, and Adhikari (2010) and Barbary et al. (2009). Flaxseeds were washed with running water for 3 min to remove the surface dust. The washed flaxseed were suspended in distilled water in a ratio 1:10 (w/v) and kept under shaking for 12 h at room temperature. The suspension was filtered through a sieve to remove the seeds and the resultant viscous solution was filtered through a double layer of cotton wool and concentrated under reduced pressure (Laborota 4002-control, Heidolph Instrument, Schwabach, Germany) at 40 °C to half volume. The mucilages were precipitated from the viscous solution by the addition of two volumes of 98% ethanol, according to the method of Cui et al. (1994), with some modifications. After allowing to stand for 1 h at 4 °C, the precipitate was collected by centrifugation (PK 131 centrifuge, ALC international, MI, Italy) at 65000 rpm for 30 min. The precipitated solid was subsequently dried in a hot air oven at 45 °C for 12 h and ground into a fine mucilage powder. The mucilage yield, with respect to the fresh seed weight, was calculated for each flax cultivar.

2.4. Solutions preparation

The mucilage powder was re-dissolved in deionized water. The solutions (0.1% or 1% (w/v)) were prepared by stirring the mucilage powder at room temperature until solubilisation was complete.

2.5. Chemical composition

2.5.1. Determination of neutral sugar

To 0.4 ml of 1% mucilage solutions (w/v in water) in a test tube were added 0.4 ml of 6 mg/ml resorcinol and 2 ml of 75% sulfuric acid. The tube was shaken by vortex and heated at 90 °C in a water bath for 30 min and subsequently placed in a cold-water bath for 30 min in the dark. The optical density of the solution was determined at 480 nm (Monsigny, Petit, & Roche, 1988) and compared to a standard solution D-xylose. The neutral sugar content was expressed as milligrammes of D-xylose equivalents per milligrammes of the mucilage powder.

2.5.2. Determination of acidic sugar

The concentration of acidic sugar was determined by a colorimetric assay following a slightly modified method from Melton and Smith (2001). 0.5 ml of 1% mucilage solution was treated with 0.5 ml of concentrated sulfuric acid. After continuous stirring for 5 min at 10 °C, the suspension was centrifuged for 10 min at 2000g at room temperature. The supernatant was considered as the mucilage hydrolysate. To 0.4 ml of the mucilage hydrolysate in a test tube, 40 µl of 4 M potassium sulfamate solution (pH 1.6) and 2.4 ml of 75 mM sodium tetraborate in sulfuric acid solution was added. The tube was mixed by vortex and heated at 100 °C in a water bath for 20 min, then placed in a cold water bath for 10 min in the dark. 80 µl of m-hydroxydiphenyl solution was added to the test tube and shaken three times by vortex. Between 10 min and 1 h after dissolution, the optical density of the solution was determined at 525 nm, with D-galacturonic acid as the standard. The acidic sugar content was calculated as milligrammes of D-galacturonic acid per milligrammes of the mucilage powder.

2.5.3. Determination of protein content

The total protein content of mucilage powder was determined by measuring the nitrogen content of mucilage powder with a CHN analyzer. A conversion factor of 6.25 was used to determine the protein content.

2.6. Physicochemical properties

2.6.1. Determination of zeta-potential, conductivity and particle size

The zeta potential of 0.1% solutions (w/v in water) was measured using Malvern Zetasizer Nano series, Nano-ZS (Malvern Instrument, Worcestershire, UK) across the disposable, folded capillary cell (DTS 1060) at 25 °C. The pH of the solutions ranged from 6.29 to 6.69. The conductivity of the samples and the particle size analysis were also measured.

2.6.2. Determination of viscosity

Viscosity of 1% solutions (w/v in water) was measured using a Brookfield Programmable DV-II Viscometer (Middleboro, MA, USA). Readings were taken at a speed in range of 0.5–2.5 rpm using spindle No.2 at 22 °C. The viscosity values were calculated in centipoise (cps) units by multiplying the dial-reading with the corresponding conversion factors provided by the manufacturer.

2.7. Functional properties

2.7.1. Solubility and swelling power

Solubility and swelling power were determined following the method described by Gebresamuel and Gebre-Mariam (2012), with a slight modification. From each mucilage sample, 25 mg was dispersed in 2 ml of distilled water in a micro centrifuge tube. The dispersion was gently shaken at room temperature (20 ± 2 °C) for 10 min. The tube was then centrifuged for 15 min at 3000 rpm. The supernatant was collected and dried to constant weight in an oven at 105 °C. The precipitated paste and the dried supernatant were then weighed. The solubility and swelling power (SP) were calculated using the following equations:

$$\text{Solubility (g/100 g mucilage)} = (M_s/M_o) \times 100 \quad (1)$$

$$\text{Swelling power (g/g mucilage)} = M_{sw}/(M_o - M_s) \quad (2)$$

where M_{sw} is the weight of the precipitated paste, M_o is the sample weight and M_s is the weight of dried supernatant.

2.7.2. Foaming properties

Foaming properties, including foaming capacity (FC) and foam stability (FS), were determined by the method of Shiahidi, Han, and Symwieki (1995) with the minor modifications made by Wang et al. (2010). Aliquots (5 ml) of 1% (w/v) mucilage solutions (pH 7.0) in a 15 ml cylinder were whipped with high-speed homogenizer (Ultra-turrax T25, IKA-Labortechnik, Janke and Kunkel IKA-Labortechnik, Staufen, Germany) at 15000 rpm for 2 min. The total volume was measured every minute after whipping until the foam volume decreased to half of its original (expanded) value. Foaming capacity was calculated as the percent increase in volume of the mucilage dispersion upon whipping, while foam stability was estimated at the time required for the foam volume to decrease to its half. Foaming capacity was calculated using the following equation:

$$\text{FC (\%)} = [(V - V_o)/V_o] \times 100 \quad (3)$$

where, V and V_o are the volumes immediately measured after whipping and before whipping, respectively.

2.7.3. Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) of the mucilages were determined according to the method of Pearce and Kinsella (1978), with minor modifications. For the emulsion formation, 5 ml of the mucilage solution and 0.5 g of soybean oil (density = 0.92 g/cm³) were homogenised using a high-speed homogenizer (Ultra-turrax T25, IKA-Labortechnik, Janke and Kunkel IKA-Labortechnik, Staufen, Germany) at 15,000 rpm

for 3 min. 100 µl of the emulsion was taken from the bottom of the homogenised-emulsion, immediately (0 min) and 60 min after homogenisation, and serially diluted to a 961 dilution (31 dilution carried out twice serially) with 0.1% (w/v) SDS solution. After manually shaking for about 5 s, the absorbance of the diluted emulsions was read at 500 nm in a spectrophotometer (8453 UV/Vis spectrophotometer, Agilent, Mississauga, Canada). The turbidity “ T value” was calculated using the following equation (Einhorn-Stoll, Weiss, & Kunzek, 2002):

$$T = (2.303 \times A \times V)/I \quad (4)$$

where T is the turbidity, V the dilution factor, A the absorbance at 500 nm, and I the path length (0.01 m).

EAI and ESI values were calculated by the following equations:

$$\text{EAI (m}^2/\text{g)} = (2 \times T)/(\Phi \times C) \quad (5)$$

$$\text{ESI (\%)} = T_1/T_0 \quad (6)$$

where Φ is the oil volume fraction of the dispersed phase, C is the concentration of flaxseed mucilage in emulsion, T_0 is turbidity immediately after emulsification, T_1 is turbidity after 1 h.

2.8. NMR spectroscopy

¹H NMR spectra, COSY (COReLation Spectroscopy), 2D TOCSY (total correlation spectroscopy) and HSQC (Heteronuclear Single Quantum Correlation) experiments were recorded in D₂O at 400.1 MHz on a Bruker AVANCE 400 Spectrometer (Bruker, Karlsruhe, Germany) equipped with a Topspin software package on a workstation running a Windows operating system. Chemical shifts (δ) are given in ppm, and were referenced to the solvent signals (δ_H 4.71). To favour complete hydrogen/deuterium exchange, samples of the isolated mucilages were dissolved in D₂O and then lyophilized three times before NMR analysis. Spectra were recorded in D₂O at ca. 4–6 mg/ml concentration. Spectra analyses were carried out using iNMR reader software (www.inmr.net) on an Apple computer.

2.9. Sensory analysis

The sensory profiling was carried out at the Sensory Laboratory at Special Company for Professional Training and Technological and Commercial Promotion of the Chamber of Commerce of Savona (Albenga, Italy). Seven subjects participated in the descriptive analysis as panellists. The subjects had variable experiences of descriptive analysis. Seven samples were prepared as 0.1% solutions in deionized water consisting of mucilage powders of different cultivars. Aliquots (20 ml) of each sample, equilibrated at room temperature (20 ± 2 °C), were presented to the panellists in a disposable plastic cup coded with random numbers. The presentation order of the samples was randomised to minimize the presentation order effect. The subjects were previously trained until a consensus was reached to evaluate the five basic tastes (sweet, bitter, sour, salty and umami). The sensory attributes were evaluated using a quantitative descriptive analysis method based on the “sip” and “spit” procedure. The subjects rinsed their mouths with filtered water before testing and between the samples. The subjects rated the intensity of stimulus using a 10-point category scale, while left and right ends were labelled “weak” and “strong”, respectively.

2.10. Statistical analysis

The experiments were run in triplicate. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and mean comparisons were run using a Duncan's multiple range tests.

Table 1

Yield, protein content and sugar content of mucilage samples prepared from different cultivars.

Cultivars	Yields* (%)	Protein content* (%)	Sugar content* (g g ⁻¹)	
			Neutral	Acidic
Valoal	1.79 ± 0.27 ^a	1.63 ± 0.06 ^a	0.56 ± 0.01 ^a	0.58 ± 0.06 ^a
Linoal	3.65 ± 0.15 ^d	1.56 ± 0.03 ^a	0.59 ± 0.00 ^{ab}	0.62 ± 0.04 ^{ab}
Merlin	3.55 ± 0.01 ^d	1.11 ± 0.04 ^b	0.55 ± 0.01 ^a	0.69 ± 0.04 ^{abc}
Kaolin	2.89 ± 0.28 ^c	1.01 ± 0.07 ^{bc}	0.77 ± 0.01 ^d	0.83 ± 0.20 ^c
Natural	2.25 ± 0.07 ^{ab}	1.23 ± 0.03 ^c	0.67 ± 0.04 ^c	0.60 ± 0.06 ^b
Festival	2.50 ± 0.48 ^{bc}	0.89 ± 0.01 ^d	0.62 ± 0.06 ^b	0.69 ± 0.04 ^{abc}
Solal	2.89 ± 0.19 ^c	0.92 ± 0.03 ^{de}	0.87 ± 0.01 ^e	0.77 ± 0.13 ^{bc}

* Values represent means ± SD from triplicate. One-way analysis of variance (ANOVA) was carried out and means comparisons were run by Duncan's multiple range tests. Different superscripts in the same column indicate significant differences ($p < 0.05$) among cultivar.

Multivariate data analysis was used to get an overview of all results from chemical, physicochemical and functional analysis. PCA (Principal Component Analysis) was applied using the chemometrics package V-PARVUS (Forina, Lanteri, Armanino, Casolino, Casale, & Oliveri, 2009).

3. Results and discussion

3.1. Mucilage yield

Flaxseed mucilage, which is a water-soluble hydrocolloid, was extracted in an aqueous solution followed by ethanol precipitation. The alcohol precipitation was used not only for the collection of dissolved polysaccharides, but also for the removal of cyanogenic glycosides, which are likely to be extracted along with the mucilage (Daun et al., 2003). The mucilage yield from seven different cultivars of whole flaxseed, using specific extraction conditions (i.e. 20 ± 5 °C extraction temperature, 12 h extraction time and 1:10 seed to water to ratio), is presented in Table 1. As shown, the flax mucilage yield ranged from 1.79% to 3.65% of fresh seed weight. The highest yields were obtained from the Linoal and Merlin cultivars (3.65% and 3.55%, respectively), whereas the minimum extraction yield was observed in the Valoal cultivar (1.80%). These values are lower than those previously reported. Barbary et al. (2009) showed that the mucilage yield can range from 3.0% to 5.2% after an extraction time of 1–8 h at 25 °C, but it increases up to 8.0% if the extraction was performed for 8 h at 100 °C. Cui et al. (1994) found that the optimum extraction can be obtained in the following conditions: temperature 85–90 °C, pH 6.5–7.0 and seed to water ratio 1:13. Since the goal of this work was to study the mucilage properties for a food or pharmaceutical industry application, we avoided the use of hard extraction conditions (i.e. high temperature) with the aim to minimize degradation products and formation of artefacts. Our results showed a significant difference in the mucilage yield was observed, highlighting that

flaxseed from different varieties contain a variable amount of mucilage. Moreover, Saag, Sanderson, Moyna, and Ramos (1975) pointed out that the extraction yield of mucilage can vary as a function of environmental factors, such as the climatic condition and crop age.

3.2. Chemical composition

3.2.1. Sugar content

The carbohydrate portion of flax mucilage can be classified into a neutral and acidic fraction. The content of the neutral and acidic fractions, expressed as milligrammes of D-xylose and D-galacturonic acid per milligrammes of the mucilage powder, respectively, is shown in Table 1. The content of both sugars varied depending on the type of flax cultivar, ranging from 0.55 (Merlin) to 0.87 (Solal) g/g for the neutral fraction and 0.58 (Valoal) to 0.83 (Kaolin) g/g for the acidic fraction. The Solal cultivar showed the richest neutral sugar fraction, while Kaolin had the highest acidic sugar fraction ($p < 0.05$). Other authors proposed that the ratio of the sugar fractions varies substantially with their origin (Izydorczyk et al., 2005). This is in agreement with our study, which found that the ratio of the neutral to acidic sugar fraction obtained from seven Italian cultivars ranged from 0.80 (Merlin) to 1.13 (Festival) (data not show). This confirms a considerable difference between the composition of flax mucilage obtained from each cultivar, which may contribute to the characterization of single mucilage.

3.2.2. Protein content

The protein content in flaxseed mucilage is relevant since it affects the interfacial properties (Fedeniuk and Biliaderis (1994)). In this study, the protein concentration of seven different cultivars has been evaluated. The lowest values, amounting to 0.89 and 0.92%, were found in the Festival and Solal cultivars, respectively, while the highest being 1.63% was present in the Valoal cultivar. The contamination with water-soluble proteins occurred during the extraction process and it represented a discriminant step in the flaxseed mucilage purification. Fekri, Khayami, Heidari, and Jamee (2008) reported that the protein content of flax mucilage can be up to 12.2% when isolation of the mucilages is carried out with distilled water (1:20 w/v) and stirring for 3 h at 75 °C. These values are higher compared with the data reported in the present study. The difference may be attributed to the use of different flax cultivar and extraction procedures. Barbary et al. (2009) pointed out that the protein content of flaxseed mucilage extracted by water at room temperature was lower than the content of samples extracted with boiling water. However, the mucilage precipitation technique with ethanol can provide the co-precipitation of other materials, such as protein, organic acids, certain salts and other similar substances (Gebresamuel & Gebre-Mariam, 2012). The proteinaceous material in mucilage samples is relevant since it affects the interfacial properties (Fedeniuk and Biliaderis (1994)).

Table 2

pH values, zeta potential, conductivity, average diameter particle size (0.1% w/v) and viscosity (1% w/v) of mucilage solution prepared from different cultivars.

Cultivars	pH	Zeta Potential* (mV)	Conductivity* (mS/cm)	z-Average diameter* (nm)	Viscosity of 1% mucilage solution* (cps)
Valoal	6.69	−17.2 ± 0.30 ^b	0.19 ± 0.0015 ^d	416.33 ± 18.82 ^a	65.0 ± 0.28 ^c
Linoal	6.70	−16.8 ± 0.47 ^b	0.13 ± 0.0000 ^b	580.00 ± 32.23 ^b	67.2 ± 0.57 ^c
Merlin	6.47	−10.5 ± 0.63 ^c	0.13 ± 0.0012 ^b	857.33 ± 130.22 ^c	133 ± 0.64 ^e
Kaolin	6.49	−19.8 ± 1.21 ^a	0.13 ± 0.0006 ^b	1031.33 ± 94.64 ^d	40.0 ± 0.21 ^b
Natural	6.40	−13.0 ± 0.83 ^d	0.14 ± 0.0012 ^c	416.00 ± 37.47 ^a	17.8 ± 0.07 ^a
Festival	6.25	−19.5 ± 0.76 ^a	0.10 ± 0.0005 ^a	559.00 ± 51.03 ^b	263 ± 1.20 ^f
Solal	6.29	−9.25 ± 0.76 ^e	0.10 ± 0.0020 ^a	325.00 ± 23.90 ^a	1211 ± 5.66 ^g

* Values represent means ± SD from triplicate. One-way analysis of variance (ANOVA) was carried out and means comparisons were run by Duncan's multiple range tests. Different superscripts in the same column indicate significant differences ($p < 0.05$) among cultivar.

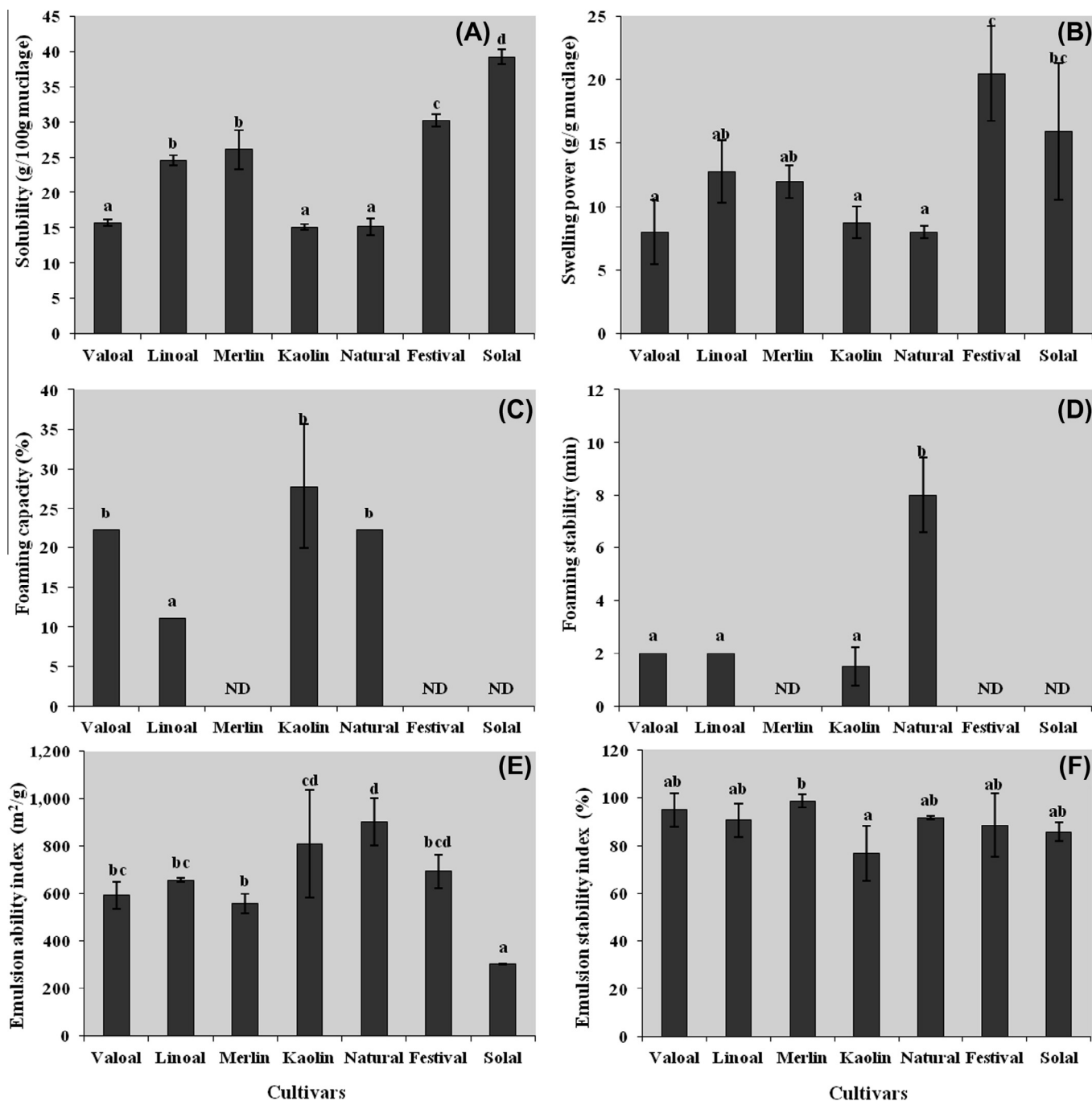


Fig. 1. Solubility and swelling power of mucilage powder (A and B); Foam capacity (FC) and foam stability (FS); (C and D); Emulsion ability index (EAI) and Emulsion stability (ES) (E and F) of mucilage solution (1% w/v), prepared from different cultivars, namely Valoal, Linoal, Merlin, Kaolin, Natural, Festival, Solal. Values of each bar with different labels were significantly different ($p < 0.05$); ND = non detected.

3.3. Physicochemical properties

3.3.1. Zeta potential, conductivity and particle size of flax mucilage solutions

Zeta potential analysis describes the stability of the colloid system. If the particles in a suspension have a large negative or positive zeta potential they tend to repel each other and no tendency for the aggregation of particles can be observed. However, if the particles have a low zeta potential value, aggregation and flocculation are favoured (Acedo-Carrillo, Rosas-Durazo, Herrera-Urbina, Rinaudo, Goycoolea & Valdez, 2006). As a general rule, the threshold between stable and unstable suspensions is generally taken at

either +30 or −30 mV (Sherman, 1970; Wang et al., 2010), although several exceptions have been described (Jones, Lesmes, Dubin, & McClements, 2010). The zeta potential, conductivity and z-average diameter of 0.1% mucilage solution particles (pH varying from 6.29 to 6.69) are presented in Table 2. All samples showed a negative charge, which ranged from −9.25 to −19.77 mV. The highest negative charge values were observed in the Kaolin and Festival cultivars, with values of −19.8 and −19.5 mV, respectively. Remarkably, the weakest negative charge values were observed in the Solal and Merlin cultivars, with the values of −9.25 and −10.49 mV, respectively. This suggests that the mucilage solutions appear to have a low stability. The zeta potential values detected in

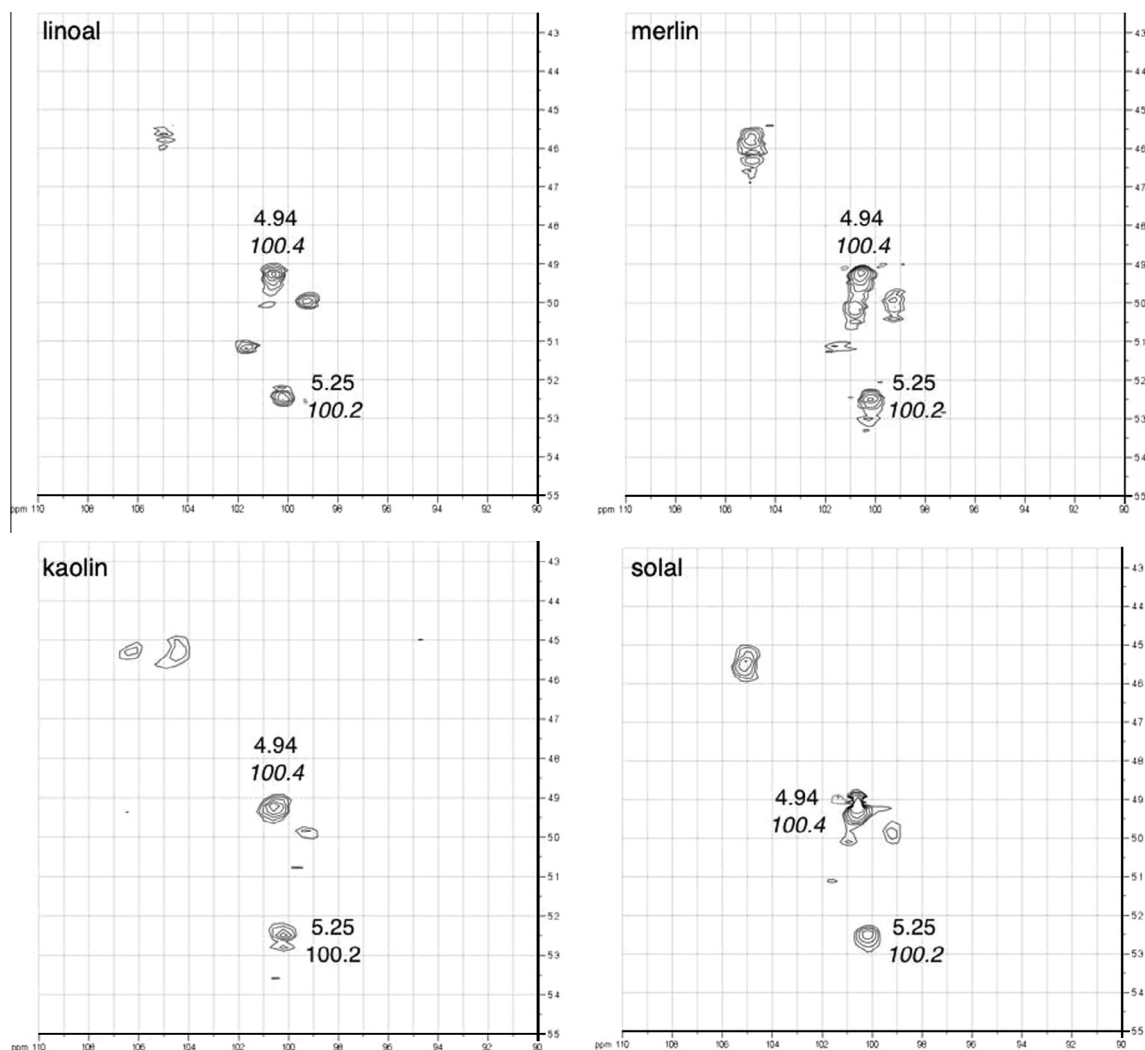


Fig. 2. Anomeric region of the HSQC spectrum of mucilage from Linoal, Merlin, Kaolin and Solal cultivar. ^1H resonances are reported in plain text; ^{13}C resonances are in italic.

our flaxseed varieties were lower than those reported by Wang et al. (2010), which were found to range between -27 and -39 mV. Since Z-potential values are the expression of structural charge densities, this discrepancy might be due to several factors that could affect the electrical characteristics of mucilage samples. The method of extraction, the content of sugar and protein and the pH value could interfere, hence inducing variations in Z-potential values. Since the range of pH was less than 0.5, it could only have a small effect on the Z-potential; hence, we could assume that the content of neutral and acidic sugars might have an effect on the variation in the Z-potential. This hypothesis is supported by the observation that the more negative Z-potential values were observed with the cultivars with a higher acidic sugar content (Kaolin and Festival).

The conductivity of the mucilage dispersions ranged between 0.1 and 1.19 mS/cm (Table 2). This variation may be attributed to the presence of ions in the solution due to the presence of electrolytes. The specific adsorption of ions onto a particle surface, even at low concentrations, exerts a dramatic effect on the zeta potential of the particle dispersion. In some cases, specific ion adsorption can lead to a charge reversal of the surface. Thus, the presence of electrolytes in the mucilage dispersions may play some role in stabilizing the suspensions.

Particle size, as a z-average diameter, of mucilage dispersions is shown in Table 2. Aqueous mucilage dispersions presented

particles with an average diameter between about 300 and 1000 nm. The highest z-average diameter, with the highest zeta potential was observed with the Kaolin cultivar, while the lowest z-average diameter, with the lowest zeta potential, was obtained from the Solal cultivar. The particle size of hydrocolloids, as well as their distribution, are important parameters concerning the rate of hydration, dissolution and emulsifying ability (Mathur, 2012).

3.3.2. Viscosity of flax mucilage solutions

The viscosity of the flax mucilage from different cultivars are reported in Table 2. Viscosity of the 1% mucilage solutions was found in the range from 17.8 to 1211 cps at 22°C . Almost all the flax mucilages exhibited relatively low viscosity, with the exception of the Solal cultivar, which showed the highest viscosity (1211 ± 5.66 cps), while the lowest value was in the Natural cultivar (17.8 ± 0.07 cps). The viscosity of a mucilage solution may depend on its chemical structure and composition. Some variability in the composition of flaxseed mucilage has been reported with respect to the cultivars and to the seasons (Oomah, & Mazza, 1998). According to Izydorczyk et al. (2005), the neutral fraction of flax mucilage mainly contributes to the high viscosity and weak gel-like properties of this gum. Indeed, the Solal cultivar showed the highest neutral sugar concentration (Table 1) and consequently the highest viscosity. Mazza and Biliaderis (1989) reported that at a concentration of 0.3% (w/v), the viscosity of flaxseed mucilage

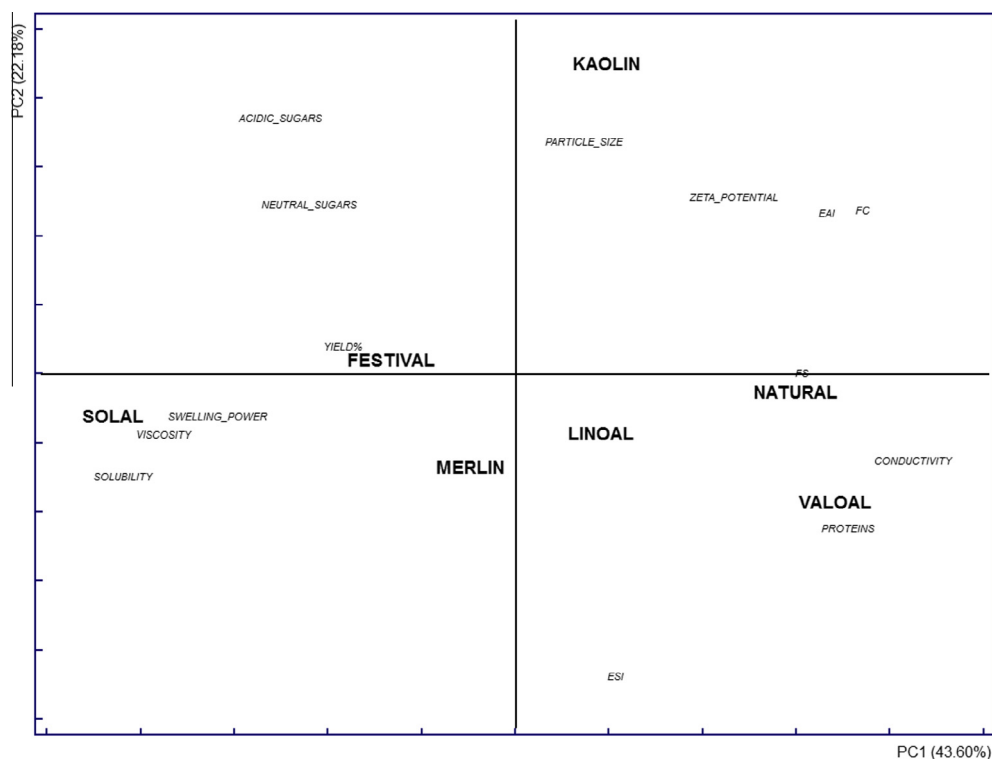


Fig. 3. PCA score (samples, in bold) and loading (properties, in italic) plot of mucilages from seven Italian cultivars of flax based on chemical, physicochemical and functional analysis.

was only about half of that of guar gum and locust bean gum, which could be changed by varying the concentration, temperature and pH. In view of these properties, mucilages of Italian flaxseed could be employed as a potential stabilizer and thickener when a high viscosity is desired, especially in the food, pharmaceutical and cosmetic industries.

3.4. Functional properties

3.4.1. Solubility and swelling power

Solubility and swelling power can be reliable descriptors of the water absorption index of mucilage powders. The solubility of the mucilages ranged between 15 and 40 g water/100 g mucilage powders when tested at room temperature (Fig. 1A). The highest degree of solubility was observed for the Solal, while the lowest were found for the Kaolin and Natural cultivar. This result is in agreement with the data of previous studies. Fedeniuk and Biliaderis (1994) reported that the water binding capacity of linseed mucilage was 16–30 g water/g sample, which was comparable to commercial guar gum (22 g/g guar gum). Moreover, it was observed that the swelling power index correlated with the solubility, i.e. the higher is the swelling power, the higher is solubility (Fig. 1B). The highest solubility (>25 g/100 g sample) and swelling power (>15 g water/g mucilage) were observed with the Festival and Solal cultivars.

3.4.2. Foaming properties

Foam capacity (FC) and foam stability (FS) of the flax mucilage solutions (1% w/v) are shown in Fig. 2C and D, respectively. Statistical analysis of the data showed that the flax cultivar had a significant effect on both FC and FS ($p < 0.05$). FC of the mucilage solutions, prepared from the Valoal, Kaolin and Natural cultivars were 20–25% capacity of foam, while the Linoal cultivar had a low FC (about 10%). In contrast, the Merlin, Festival and Solal cul-

tivars showed no foaming properties after 2 min of whipping (Fig. 1C). The FC was lower than xanthan and guar gum solutions (0.5%), which were reported to be 15% and 27%, respectively, when tested using a similar method (Sciarini, Maldonado, Ribotta, Perez, & Leon, 2009). Fig. 1D shows the foam stability of mucilage solutions. All the mucilage solutions maintained their volumes for only short time, ranging from 1 to 10 min with significant differences between different cultivars ($p < 0.05$) (Fig. 1D). The Natural cultivar had the highest FS, whereas the Valoal, Linoal and Kaolin cultivars had very low foam stabilities. Indeed, the foam reduced to a half within 2 min after whipping. In general, a key factor conferring both ease of preparation and stability of foams is the nature and mobility of the surfactant molecules at the two-phase interface (Richmond, 1992). The very low foaming properties of some mucilage samples could be due to a very low alignment of the mucilage polysaccharides at the air-water interface. Although polysaccharide gums are not generally considered as surface-active agents, it has been shown that they can increase the foaming properties of protein systems, increasing bulk-phase viscosity and creating a biopolymer network that prevents air-bubble coalescence (Makri & Doxastakis, 2007). In addition, the presence of a small amount of proteinaceous material in the mucilage may be associated with the interfacial properties (Fedeniuk & Biliaderis, 1994).

3.4.3. Emulsifying properties

The emulsion activity index (EAI) and emulsion stability index (ESI) of the 1% mucilage solutions from different flax cultivars are shown in Fig. 1E and F, respectively. The EAI of all the mucilage samples ranged from 300 to 900 m²/g. The highest EAI values were observed in the Kaolin and Natural cultivars, while the lowest was obtained from the Solal cultivar (Fig. 1E). The EAI values in this study were much higher than those reported by Wang et al. (2010), in which the values are indicated as 70 m²/g, regardless of the flax cultivars. A number of reports found that the ethanol

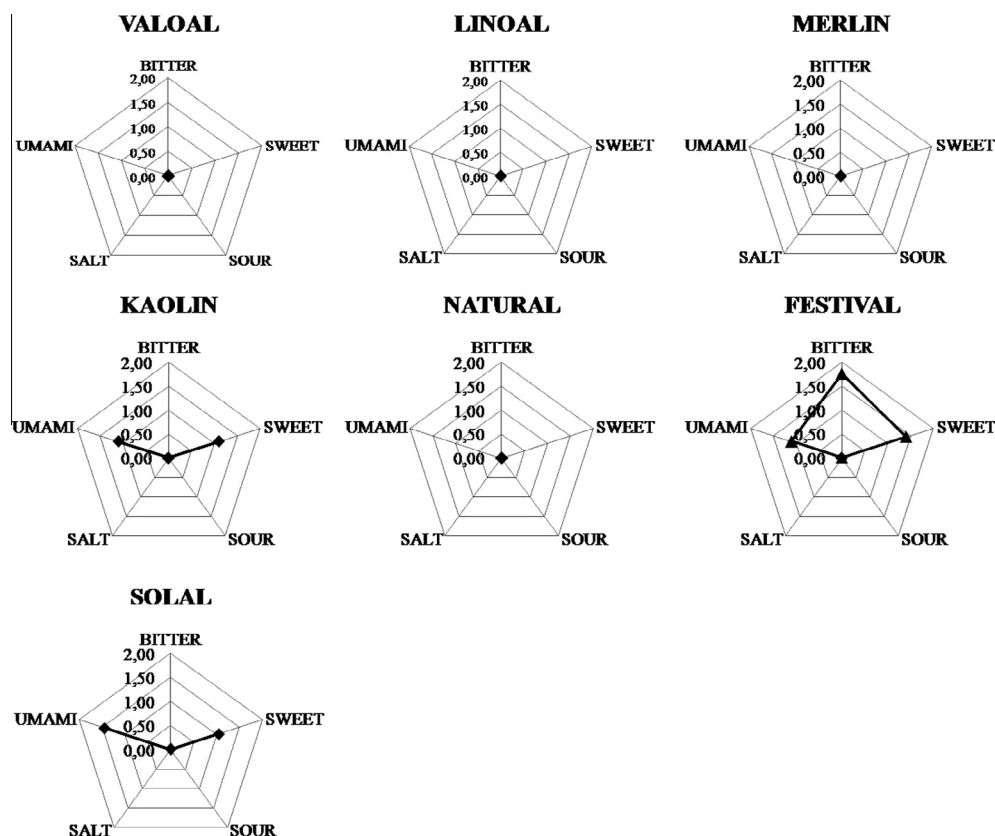


Fig. 4. Taste profiles of mucilage 0.1% w/v solution, prepared from different cultivars, namely Valoal, Linoal, Merlin, Kaolin, Natural, Festival, Solal. Each value represents the averaged taste intensity scored on 10-point category scale by at least 5 panellists. Results are represented with a reduced scale for easier interpretation.

precipitation method appears to be the best to produce mucilage powder with best emulsion properties, compared with the other drying method (Wang et al. (2010)). All the mucilage samples showed a high stability of emulsion lasting 60 min after whipping (Fig. 1F). The ESI of all the mucilage samples showed more than 80%, excepted for Kaolin, which was found to be 76.8%. Mathur (2012) explained that polysaccharide hydrocolloids stabilize emulsions primarily by increasing the viscosity and also by acting as an emulsifier, where their emulsification ability is reported as mainly being due to contaminating or intrinsic protein material present in them. In particular, the electrostatic interaction between ionic hydrocolloids and proteins gives rise to a marked emulsification ability with considerable stability at the appropriate pH and ionic strength (Mathur, 2012).

3.5. NMR spectroscopy

Although the structural elucidation of the flax mucilages is beyond the scope of this work, it seemed nonetheless interesting to make a rough comparison of the structural features of the polymers from the different flax cultivars by recording NMR spectra of the crude mucilage samples obtained by water extraction without further purification.

It is known that rhamnogalacturonans are among the most abundant and structurally complex polysaccharides present in plant cell walls, including flax seeds (Naran, Chen, & Carpita, 2008), and details of their structures are highly variable in polymers from different sources (Mikshina, Gurjanov, Mukhitova, Petrova, Shashkov, & Gorshkova, 2012; Nascimento et al., 2013).

Inspection of the ^1H NMR spectra of all the samples analysed showed a signal at 1.25 ppm, attributable to the methyl group of rhamnose residues.

No signals were present in the region between 2.0 and 2.2 ppm, thus indicating the lack of acetylated sugars (Bédouet, Courtois, & Courtois, 2005).

In the HSCQ spectra of the mucilages obtained from all the cultivars, a series of signals attributable to anomeric protons in the range 4.43–5.41 ppm, correlating with the anomeric carbons between 97.5 and 103.3 ppm were present. Among these, a signal at 5.25 ppm, whose carbon atom resonates at 100.2 ppm, could be assigned to the rhamnose residues of a rhamnogalacturonan backbone. The NMR spectra obtained from the Linoal cultivar, as a representative example, and starting from the signal at 5.25 ppm, TOCSY and COSY spectra analysis allowed for all the resonances of rhamnose to be identified through the correlations of peaks signals at: δ 5.25, 4.11, 3.86, 3.39, 3.77 and 1.25 (Mikshina et al., 2012).

Another common signal in the NMR spectra of all the mucilages was a proton at 4.94 ppm, correlating with a carbon atom at 100.4 ppm, presumably due to the galacturonic acid residues of the polysaccharides backbone. In addition, some peculiar signals were found in the HSQC spectra of each mucilage, whose intensities and chemical shifts were highly variable. Thus, these are presumably due to the different sugar residues in the side chains of the polysaccharides (Fig. 2), whose structures are under investigation and will be published in due course.

3.6. PCA for an overview of the results

In order to get an overview of all the results and to explore relationships between chemical, physicochemical and functional data for each flax sample, PCA was performed with. Using PCA, the first two principal components could be described 65.8% of the variation in the data set. In the PCA score and loading plot (Fig. 3)

sample grouping can be studied. It is possible to notice that the first principal component, which is the direction of the maximum explained variance (43.6%), demonstrated a useful separation between the samples. From the loadings it is seen that samples placed to the left along PC1 (i.e. Solal and Festival cultivars) are characterised by high viscosity, solubility and swelling power, as well as by a high neutral sugar content, and at the same time they showed low protein content and emulsifying and foaming properties. In contrast, samples placed to the right along PC1 (i.e. Natural and Valoal varieties) are characterised by good functional properties (FC and FS, EAI). Moreover, PC2, which explains a lower variance percentage (22.2%), reveals a total separation of Kaolin from all the other flax sample. This variety seems to be characterised by the highest zeta potential absolute value and average particle size and good acidic sugars content and emulsifying and foaming properties. From these results, it was possible to confirm also that viscosity was closely correlated to the neutral sugar content and zeta potential was linked with the average particle size, while functional properties were associated to the presence of proteinaceous materials, as previously described. From the loading plot it was possible also to define that ESI and mucilage yield were not characteristic parameters for sample mucilage characterization.

3.7. Sensory analysis

Physicochemical and functional properties of flaxseed mucilages described in the previous paragraphs, suggest a potential use as food additives in the food industry. Texture is of major importance for consumers acceptability of food products. In order to study the sensory characteristics of the mucilage samples obtained from different cultivars, 0.1% solutions of each product were tested by a team of selected and certified panellists. Fig. 4 shows the average taste intensity of the five main flavours for the seven Italian cultivars studied. All descriptors were the same among flaxseed cultivars, with the exception of bitter, sweet and umami taste for the Kaolin, Festival and Solal cultivars. The Valoal, Linoal, Merlin and Natural varieties were tasteless, while the Kaolin, Festival and Solal cultivars showed a low sweet and umami taste, less than 2 points on a 10-point category scale. Only the Festival variety mucilage showed a low bitter taste. The little taste of flax mucilage is in agreement with previous studies related to the use of vegetal gums as edible food additives or coating (Del-Valle, Hernández-Muñoz, Guarda, & Galotto, 2005; Koocheki, Mortazavi, Shahidi, Razavi, & Taherian, 2009). From these results, it seems that flaxseed mucilage do not have a direct influence in the natural taste and flavour of foodstuffs. This is an important aspect regarding the use of food additives when taste modification is undesirable.

4. Conclusion

This study provides useful information regarding flaxseed mucilage chemical features for their potential industrial application. The availability of a mucilage fully characterised endowed with different chemical, physico-chemical and functional properties provides a tool-set for practical application in several sectors, ranging from the food to pharmaceutical and cosmetic industry. In addition to the chemical characterization, the suspension stability, the wide range of viscosity, the good water absorption and the different foaming and emulsifying properties observed among the studied cultivar open the chance to use these products as potential new food additives. Particularly, the evidence that all mucilage samples were tasteless or had a negligible taste profile, can address the use of these samples in food products without affecting the taste of the product itself.

In particular, the Solal and Festival cultivars appeared to be suitable as thickeners in food processes due to their high viscosity, good solubility and swelling power, whereas the Natural, Valoal and Kaolin could find an application as emulsifiers for their good surface-active properties.

Further work will be done to give more insight in the structural features responsible for the variability observed among the studied cultivar. The monosaccharide composition of the carbohydrates and additional NMR analysis will be performed with the aim of correlating the chemical, physicochemical and functional properties of the flaxseed mucilages with the structural characteristics of their constituents.

Conflicts of interest

The authors declare no financial or commercial conflict of interest.

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