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Flavor of fava bean (*Vicia faba* L.) ingredients: Effect of processing and application conditions on odor-perception and headspace volatile chemistry

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ABSTRACT

Application of plant-based sources for food, e.g. fava bean, is challenged by consumer acceptance. This study attempts to understand how ingredient processing and application conditions drive fava bean flavor. An approach to evaluate odor perception along with the analysis of headspace volatile compounds detected during ingredient utilization was used. Precisely, a protein-rich ingredient, i.e. air classified fava bean concentrate, selected for its high industrial potential, was modified by pH (2, 4, 6.4 and 11), temperature (55, 75 and 95 °C) and treatment duration (30 and 360 min). The experimental design produced 36 different modified ingredients, which were further subjected to two distinct models of beverage application (pH 4 and 7). Results showed that the “green” perception detected in the initial concentrate evolved more into “cooked” perception with ingredient processing. Application conditions drove aroma changes, ranging from a “sweet” to “rancid” perception when changed from neutral to acidic pH. Aldehydes were generated in many ingredients, as well as furanoids at pH 2, terpenoids at pH 4, alcohols at pH 6.4 and ketones at pH 11. Lipid oxidation was hypothesized as the major contributor to the aroma composition in the ingredient suspensions. Reactions involving protein, sugar and carotenoid degradation, including Maillard reaction and caramelization, also played a role in the flavor generation. Different suspension matrices at different pH during ingredient application might have influenced the release of pH-dependent volatiles. This data allows to better link the role of process conditions in the generation and release of hypothesized odor-active molecules associated with different odor sensory notes. Thus, various flavor profiles can be driven by process conditions for fava bean concentrates – making it promising for several food applications.

1. Introduction

The demand for healthy foods is rising and by 2050 a global sustainable transformation in the food system is essential to be able to feed a world population of nearly 10 billion people (Willett et al., 2019). Fava bean (*Vicia faba* L.) is a promising protein source for human consumption. It has a great agronomic potential owing to a higher crop yield per harvest area and growth at temperatures as low as 12.5 °C. Also, by virtue of its high protein content, i.e. 23–41% (w/w) d.b., there is a large nutritional and functional potential for food applications (Multari, Stewart, & Russell, 2015; Sharan, Zanghelini, et al., 2021). Nevertheless, there are sensory (flavor and color) and anti-nutritional limitations that need to be addressed to increase consumer acceptability of such foods (Sharan, Zanghelini, et al., 2021). Fava bean is often processed to

form ingredients (flours, concentrates and isolates) that can be further modified using process conditions (ingredient modification) to eventually increase their utilization in industrial food applications (ingredient utilization) (Boye, Zare, & Pletch, 2010; Sharan, Zanghelini, et al., 2021). These processing steps can drive a generation of undesirable flavor compounds or, conversely, limit the presence of off-flavors in certain conditions (Roland, Pouvreau, Curran, Van De Velde, & De Kok, 2017).

Flavor perception of foods is a complex process that involves the senses of smell and taste, and chemesthesis, which require the interactions between non-volatile and volatile molecules with sensory receptors in the oral and nasal cavities (Roland et al., 2017). Concerning sensory properties of pulses, the unpleasant bean flavor is often linked to (i) the perception of green, grassy and beany notes, which are mainly

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attributed to aldehydes, alcohols and ketones, and (ii) a bitter and astringent taste which is associated with sapid-glycosylated compounds such as saponins and phenolic compounds (isoflavones, flavonols, phenolic acids, etc.) (MacLeod, Ames, & Betz, 1988; Damodaran & Arora, 2013; Ong & Liu, 2018). Odor is one of the first key indications of flavor in foods, reflecting their quality and acceptability (Lawless, 1991; Herz, 2016; Thomas-Danguin, Barba, Salles, & Guichard, 2016; Spence, 2021). The intrinsic aroma of a plant depends on its genetic makeup, but also on the availability of flavor precursors, the distribution of enzymes and the presence of favorable conditions for reactions to take place (Roland et al., 2017; Ong & Liu, 2018). While a minor part of the fava bean volatiles manifest during bean development, a majority of them derive from degradation of lipids, amino acids, carbohydrates and carotenoids through enzymatic and/ or non-enzymatic reactions (Reineccius & Heath, 2006). These reactions are prone to occur throughout the ingredient supply chain, i.e. from bean harvest until final food application (Roland et al., 2017; Sharan, Zanghelini, et al., 2021).

Lipid oxidation is the primary cause of flavor generation in pulses. Numerous studies regarding pea flavor have shed light on many lipid oxidation products such as aldehydes, ketones, alcohols and pyrazines, which induce a combination of green, beany, earthy and hay-like sensory notes (Malcolmson et al., 2014; Roland et al., 2017). Fava bean flavor reactions have also been investigated, but to a lesser extent and not particularly in direct correlation with sensory attributes. In fava bean, free or esterified unsaturated fatty acids can undergo enzymatic reaction by broad bean lipoxygenase (BBL) or auto-oxidation due to the presence of initiators (e.g. light, chlorophyll, metal ions) and/ or high temperatures (Sharan, Zanghelini, et al., 2021). Other reactions that can lead to additional flavor development include amino acid and sugar degradation, along with their rearrangement by Strecker degradation and Maillard reaction (MacLeod et al., 1988; Reineccius & Heath, 2006). Process conditions largely impact flavor, as they influence the extent and possibility of flavor-associated reactions and thus govern flavor formation. For fava bean, the impact of process conditions on the bean flavor itself has been studied to a certain extent. Fava bean seeds under microwave treatment (950 W for 1.5 min) or heat treatment (> 70 °C, > 2 min) give modified flavor compared to fresh beans due to the inactivation of BBL (Eskin & Henderson, 1974; Al-Obaidy & Siddiqi, 1981; Jiang et al., 2016). Fava bean ingredients, including flours that are dehulled and milled, contain high BBL activity, thus suggesting possibilities of enzymatic lipid oxidation (Yang, Piironen, & Lampi, 2017). The effect of pH has also been tested on fava bean isolates for flavor modification – in which dried pea-like flavor predominated at neutral pH and unpleasant fruity flavor developed at acidic pH (Schultz, Hoppe, & Schmandke, 1988). Despite some understanding on fava bean flavor, there is a need of a comprehensive knowledge of the chemistry of fava bean flavor with process conditions and its relation to sensory perception. In this way, the food industry can choose the right kind of processing for a target food application with controlled aroma perception, thus increasing acceptability for the use of fava bean as promising food ingredients.

Fava bean concentrate produced by air-classification is identified as a gently processed ingredient (Tamayo Tenorio, Kyriakopoulou, Suarez-Garcia, van den Berg, & van der Goot, 2018). In this study, this ingredient was modified by various process conditions before its utilization in model food. The impact of industrially relevant process conditions such as pH, temperature and treatment duration was established on two main aspects: (1) odor perception, i.e. qualitative and quantitative sensory properties, and (2) volatile chemistry, i.e. headspace release of volatiles in conditions close to beverage application. Relationships were established to understand the interplay between processing, flavor generation and volatile chemistry in producing different ingredients with diverse flavor profiles. With this, their potential in a variety of food applications could be foreseen.

2. Materials & methods

2.1. Ingredient preparation

2.1.1. Fava bean initial concentrate (FBIC)

Fava bean concentrate containing 65% (w/w d.b.) proteins was produced by Döhler GmbH (Darmstadt, Germany). The concentrate was produced by milling of dried and dehulled beans followed by air classification (Felix, Lopez-Osorio, Romero, & Guerrero, 2018).

2.1.2. Modified ingredients

The FBIC was modified as described below: 20% (w/w) suspensions were prepared with Milli-Q water (Millipore, Burlington, Massachusetts, United States) and stirred for 30 min at 500 rpm using an overhead dissolver stirrer (IKA Works, Inc., Staufen, Germany); the pH (pH_{process}) was then adjusted to 2, 4 or 11 using 6 mol/L hydrochloric acid or sodium hydroxide (Sigma Aldrich, St. Louis, Missouri, United States), and the suspensions were further stirred for 30 min at 500 rpm. Additionally, a series with the natural suspension pH was also considered (pH_{process} 6.4) and further stirred for 30 min at 500 rpm. All the suspensions were then heated (T_{process}) in a temperature-controlled bath (Lochner Labor + Technik GmbH, Berching, Germany) maintained at 55, 75 or 95 °C and agitated at 700 rpm for a duration (t_{process}) of either 30 min (Low) or 360 min (High). All the treatments at pH_{process} 4 were performed in triplicates in order to assess reproducibility. The different suspensions that were produced were further frozen at – 20 °C, followed by freeze-drying (Döhler GmbH, Dahlenburg, Germany) and milling to 0.08 mm mesh size by an ultra-centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany). Hence, different modified ingredient powders were obtained, which were named as $pH_{\text{process}}-T_{\text{process}}-t_{\text{process}}$ (e.g. pH2_55 °C_Low) based on the conditions used to modify them.

2.2. Odor sensory profiling

Sensory odor profiling of the different ingredients was performed using three stages after panel recruitment: a) attribute generation and selection, b) panel training using references for each attribute and c) evaluation of odor description and intensity profiling of the main odor key notes for all the samples.

2.2.1. Sample preparation & presentation

For the sensory evaluation, all ingredients (FBIC and modified ingredients) were suspended to 5% (w/w) powder concentration in deionized water and stirred for 30 min at 20 °C. Furthermore, the pH of these suspensions was readjusted to $pH_{\text{utilization}}$ (4 or 7) using 6 mol/L hydrochloric acid or sodium hydroxide, respectively (Sigma Aldrich, St. Louis, Missouri, United States). The total weight of the suspension was 5 g per panelist. The references and products suspensions were presented to the panelists in 80 mL plastic cups covered with a lid. The samples were labeled with random three-digit numbers and served to the panelists at room temperature within 30 min after the preparation of the product suspensions. During each round of analysis, each participant received 12 products served on a tray in the order specified by Latin Square experimental design to account for possible carry-over effects.

2.2.2. Subjects

Twenty-one volunteer panelists (13 women and 8 men, 18–40 years in age) were recruited based on their ability and willingness to participate in this study. The panelists had previously different levels of experience in sensory study participation. The overall aim of the experiment was communicated to all panelists beforehand; they gave their free and informed consent and additionally received compensation for their participation. Prior to the sessions, the panelists were asked not to smoke or consume coffee, tea or other flavor-intense foods. All sessions were

conducted and monitored by the authors. The experimentation was performed at UMR SayFood (Université Paris-Saclay, INRAE, AgroParis-Tech, on the sites of Grignon & Massy, France). All communication was in French.

2.2.3. Panel training

The panel training was conducted with three objectives: a) to memorize different odor attributes with the references provided; b) to reach a consensus between all the judges on the choice of the attributes to use and their definition; and c) to train to evaluate the key odor by selection of the main notes characterizing the samples and evaluation of the perceived intensities of a reduced number of attributes. The complete list of attributes was selected after discussion with the panelists to describe all the perception of the ingredients, and various reference products were proposed to the subjects to help with recognition and learning of various sensations. After the training sessions, a total of 36 attributes was finally selected for the subsequent sessions (Table 1) with a Check-All-That-Apply (CATA) test. Additionally, four different classes of attributes were identified by the judges to possibly categorize the 36 chosen attributes, and to define their perceived intensities in the different samples presented: green (*notes vertes*), “sweet” (*notes sucrées*), rancid (*notes rances*) and cooked notes (*notes cuites*). The quoted lexicon “sweet” has been used to describe the aroma of beverages (e.g. brewed coffee), in which the term is associated with caramel/vanilla aroma notes (Chambers et al., 2016). The intensities of 4 odor classes, along with CATA analysis of the 36 different chosen attributes were evaluated and subsequently associated using statistical tools (See Section 2.4.3).

2.2.4. Odor attribute profiling & intensity scaling

Sample evaluation sessions were conducted after the training sessions, using the LimeSurvey platform (LimeSurvey GmbH, Hamburg, Germany). Each sample was evaluated by two methods: a) selection of most pertinent attributes for the sample using CATA method (Table 1); and b) perceived intensity scaling ranging from 0 (none or negligible perception) to 10 (very intense) of the four principal notes identified: green, “sweet”, rancid and cooked notes.

The tests were carried out in single replicate. As ingredients modified by pH_{process} 4 were produced in triplicates, odor analysis of these true replicates was assessed for reproducibility.

2.3. Flavor volatile chemistry

2.3.1. Internal deuterated standard

For each sample preparation, an alcoholic stock solution containing 0.1 µg/mg d7-heptanol (Cluzeau Infor Labo C.I.L., Sainte-Foy-la-Grande, France) was prepared and stored at -20 °C until use.

2.3.2. Sample preparation for volatile compounds analysis

The 2 g mixtures of 10% (w/w) ingredient suspensions were prepared using the following method: the ingredients (FBIC and modified ingredients) were suspended in triplicates in deionized water in 20 mL SPME vials, which were immediately sealed with aluminum polytetrafluoroethylene coated silicone septum caps in order to avoid loss of volatiles. The vials were agitated by IKA Vortex 2 (IKA Works, Inc., Staufen, Germany) for 30 min at 20 °C, followed by a pH adjustment of either pH_{utilization} (4 or 7) using 0.1 mol/L hydrochloric acid or 0.1 mol/L sodium hydroxide, respectively (Sigma Aldrich, St. Louis, Missouri, United States). Additionally, 100 ng of d7-heptanol from the stock solution was introduced into the vials after the pH adjustment. The required amounts of acid, base and standard stock were added into the vials using a 50 µL eVol™ syringe (Trajan Scientific and Medical, Ringwood, Australia).

Table 1
Check-All-That-Apply (CATA) Odor Sensory Evaluation of Fava Bean Ingredients.

Attributes in English	Attributes in French	Reference
Cut Grass	<i>Herbe coupée</i>	10% w/w (Z)-3-hexenol (CAS: 928-96-1) in ethanol
Celery	<i>Céleri</i>	Cubes of fresh cut celery
Hay	<i>Foin</i>	Horse hay from experimental farm (Grignon, France)
Lentil	<i>Lentille</i>	Liquid from canned lentils (Auchan, France)
Potato	<i>Pomme de terre</i>	Liquid from canned lentils (Auchan, France)
Mung Bean	<i>Haricot mungo</i>	Liquid from canned mung bean sprouts (Auchan, France)
Fresh	<i>Frais</i>	10% w/w L-menthol (CAS: 89-78-1) in ethanol
Wood	<i>Bois</i>	Bits of tree barks (Grignon, France)
Earthy	<i>Terre</i>	Moist soil (Grignon, France)
Spices	<i>Epice</i>	Four spices mix (Auchan, France)
Caramel	<i>Caramel</i>	Caramel sauce (Vahiné, France)
Rancid	<i>Rance</i>	Vegetable oil stored for several years (Grignon, France)
Grilled	<i>Grillé</i>	Grilled almonds (Auchan, France)
Burnt	<i>Brûlé</i>	Almonds grilled until black (Grignon, France)
Smoky	<i>Fumé</i>	Smoky barbecue sauce (Auchan, France)
Coffee	<i>Café</i>	Arabica coffee powder (Auchan, France)
Chocolate	<i>Chocolat</i>	Cocoa powder (Auchan, France)
Hazelnut	<i>Noisette</i>	Whole hazelnuts (Auchan, France)
Coconut	<i>Coco</i>	Grated coconut (Auchan, France)
Orange Blossom	<i>Fleur d'oranger</i>	Orange Blossom Aroma (Fabster, France)
Vanilla	<i>Vanille</i>	10% w/w ethyl vanillin (CAS: 121-32-4) in ethanol
Banana	<i>Banane</i>	Concentrated banana aroma (Fabster, France)
Almond	<i>Amande</i>	Almond oil (Auchan, France)
Citrus	<i>Citron</i>	Fresh cut pieces of lemon
Red Wine	<i>Vin Rouge</i>	Red wine ‘Les Fiefs de Lagrange’ (Saint-Julien, France)
Vinegar	<i>Vinaigre</i>	Vinegar (Auchan, France)
Milk	<i>Lait</i>	Whole milk (Lactel, France)
Butter	<i>Beurre</i>	10% w/w 2,3-butanedione (CAS: 431-03-8) in ethanol
Cream	<i>Crème</i>	Whole milk fresh cream (Yoplait, France)
Egg	<i>Oeuf</i>	Hard boiled eggs, peeled and cut (Auchan, France)
Meat	<i>Viande</i>	Beef bouillon (Auchan, France)
Ammoniac	<i>Ammoniac</i>	10% w/w ammoniac (CAS: 7664-41-7) in ethanol
Soap	<i>Savon</i>	Unscented soap bar (Le Petit Marseillais, France)
Chemical	<i>Chimique</i>	10% w/w ethyl acetate (CAS: 141-78-6) in ethanol
Cigarette	<i>Cigarette</i>	Nil [§]
Petrol	<i>Pétrole</i>	Nil [§]

Final list of attributes used for CATA test and associated references used for panel training. These attributes were used to describe different fava bean ingredients modified by pH: 2, 4, 6.4 and 11 (pH_{process}), temperature: 55, 75 and 95 °C (T_{process}) and treatment duration: 30 and 360 min (t_{process}), and then utilized at application pH: 4 and 7 (pH_{utilization}).

§ - This attribute is well known by the panelists, and the reference product for it is avoided due to safety and sensory reasons (Mehlman, 1992; Starek & Podolak, 2009; Fjældstad, A; Fernandes, 2020; Pullicin et al., 2020).

2.3.3. Extraction of volatile compounds from the headspace

The volatile compounds were extracted from the headspace of the samples by automated solid-phase micro-extraction (HS-SPME). Prior to extraction, each vial was incubated at 50 °C for 36 min under agitation (10/1 s on/off) to reach equilibrium between the matrix and the headspace. For the ensuing extraction, a gray-notched divinylbenzene/carboxen/polydimethylsiloxane DVB/CAR/PDMS 2 cm fiber (Supelco Inc., Bellefonte, Pennsylvania, United States) was exposed to the headspace for 42 min at the same temperature. The fiber was then desorbed on a GC injection port, which was held at 250 °C, during 2 min. A

10 min fiber reconditioning procedure was performed at 270 °C between samples. The DVB/CAR/PDMS fiber was selected to ensure an efficient extraction of a wide range of volatile compounds (Barra et al., 2007; Mishra, Tripathi, Gupta, & Variyar, 2017; Cepeda-Vázquez, Rega, Descharles, & Camel, 2018).

2.3.4. GC-MS semi-quantitative analysis of volatiles

The headspace extracts were analyzed by gas chromatography (GC) in a Trace GC Ultra system coupled to an ISQ single quadrupole mass spectrometer (MS; Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States). A non-polar ZB-5MSPLUS column (30 m × 0.25 mm × 0.25 µm; Zebron, Anaheim, California, United States) was chosen for separation. Helium was used as carrier gas at a constant flow rate of 1.2 mL/min. The parameters were based on the method optimized by Cepeda-Vázquez (2017) in the same equipment, with slight adjustments following pre-tests to achieve a better chromatographic separation of the volatile compounds present in faba beans. Injections were done in splitless mode. The GC oven was programmed as follows: initial temperature of 40 °C (held for 5 min), then raised at 1 °C/min until 90 °C and 15 °C/min until a final temperature of 240 °C (held for another 5 min). Mass spectrometry was carried out using electron ionization at 70 eV. MS transfer line and ion source temperatures were set to 250 °C and 200 °C, respectively. A standard solution of deionized water with 100 ng d7-heptanol was analyzed for each sequence of 10 runs to assure the steadiness of the system's response over time. Data acquisition was done in full scan mode (TIC, m/z 33–300). Each compound was identified by comparing its experimental mass spectra with the Wiley 8 and NIST 08 (National Institute of Standards and Technology 2008) mass spectral libraries using Xcalibur 2.1.0 (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States). Only compounds with an SI match factor above 800 (out of 1000) were retained and their retention indices obtained from a series of n -alkanes (C5–C21) were further compared with the values reported on the NIST database. The retention indices were calculated using the isothermal and non-isothermal formulae established by Kovatz (Eq. (1)) and Van den Dool and Kratz (Eq. (2)), respectively, which are based on the retention times of a series of alkanes (C5–C17, C19–C23) analyzed under the same conditions. Eq. (1) was only applied in the peaks eluted in the first and last 5 min of analysis, for which the oven temperature was held constant, while Eq. (2) was used for all additional peaks.

$$I_x = (100 * n) + 100 * \frac{(\log t_x - \log t_n)}{(\log t_{n+1} - \log t_n)} \quad (1)$$

$$I_x = (100 * n) + 100 * \frac{(t_x - t_n)}{(t_{n+1} - t_n)} \quad (2)$$

where I_x = retention index of the volatile, t_x = retention time of the volatile, t_n and t_{n+1} = retention times of the smaller and larger alkanes corresponding to the volatile.

To ensure a robust semi-quantification of the detected volatiles, the integrated volatile peak areas above the limits of quantification were selected by filtering those with peak heights above 10 times the height of baseline noise. The chromatographic peak areas for each compound were extracted using quantifier ions specific to the compound, then integrated using the Quan browser of Xcalibur 2.1.0 (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States). Such a technique of extracting TIC areas by selected ions has been known to improve peak quality and resolution, especially for individual volatile variations between a set of samples (Salvadeo, Boggia, Evangelisti, & Zunin, 2007; Perez-Hurtado et al., 2017; Qiu et al., 2018). The peak areas were then normalized with d7-heptanol of their respective chromatograms and the ingredient dry weights measured for each chromatographic analysis (Lee et al., 2020).

2.4. Data analysis

Statistical data analysis was conducted using XLSTAT 2021.1. (Addinsoft, Paris, France).

2.4.1. Analysis of odor profile

A matrix of the selected attributes from CATA data across all samples and judges was obtained in a binary form (0 or 1). First, Coqran's Q test ($p \leq 0.05$) was used to identify the attributes that significantly discriminated the ingredient samples, followed by the Critical difference (Sheskin) multiple pairwise comparison between the attributes. Correspondence analysis (CA) with Chi-square distancing was conducted on these significant attributes across all judges and samples.

For the perceived intensities, analysis of variance (ANOVA) with post-hoc treatment using Newman-Keuls (SNK) method ($p \leq 0.05$) was performed across all the samples and judges. Furthermore, means of the intensities noted across all judges for each ingredient suspension was created and a Principal Component Analysis (PCA) by Pearson's correlation method was conducted on this matrix.

2.4.2. Analysis of volatile profile

A matrix of the normalized peak areas of all quantifiable volatiles was obtained across all ingredient suspensions. ANOVA with post-hoc treatment using Newman-Keuls (SNK) method ($p \leq 0.05$) and PCA Pearson's correlation method was conducted on this matrix.

2.4.3. Association between processing, odor perception and volatile chemistry

The relationships between the composition, the odor characteristics and intensities, as well as the relative amounts of volatile constituents (those with an expected odor activity from literature data/studies) of different ingredient suspensions (Arn & Acree, 1998; Garg et al., 2017), were examined using Multiple Factor Analysis (MFA). Four qualitative variables (pH_{process} , T_{process} , t_{process} , $pH_{\text{utilization}}$), along with three quantitative matrices (CATA data, average odor intensities and normalized volatile peak areas) were used for the MFA. Summation of the binary CATA data for each sample across all judges was prepared exclusively for this analysis.

3. Results

3.1. Odor perception of fava bean ingredients

3.1.1. Panel performance

The panel performances were first evaluated and controlled considering repeatability, discrimination and homogeneity criteria of sensory profiling results. Overall performances were assessed using ANOVAs with three independent variables (product type, subjects, and replicate) and their first-order interactions (Table 2). This three-way ANOVA was performed on the odor attribute intensities (green, "sweet", rancid and cooked notes) evaluated on a selected group of fava bean modified ingredients, i.e. the pH_{process} 4 series, which was performed in triplicate. A significant product effect was observed for 3 out of 4 attributes (except for the cooked note), indicating that the panelists distinguished among the different samples ($p < 0.05$). The significance of the first order interactions indicated whether the panelists consistently scored attributes across replicates (subject * replicate), if there was consistency in scoring different ingredient suspensions among the panelists (product type * subject), and whether ingredient suspensions behaved consistently across replicates (product type * replicate). At first, the interaction between the replicate and product type (product type * replicate) was not significant for all attributes (Table 2). With a considerable decrease in the F-value, the subject and product type interaction remained significant for all attributes, suggesting a possible heterogeneity of evaluation between panelists or an effect of different uses of the scale between pan-

Table 2
Odor sensory panel performance of fava bean ingredient suspensions.

Attribute	Subject		Replicate		Product Type		(Subject * Replicate)		(Subject * Product Type)		(Replicate * Product Type)	
	F	p-Value	F	p-Value	F	p-Value	F	p-Value	F	p-Value	F	p-Value
Green	37.3	<0.0001	3.5	0.032	2.0	0.029	2.8	<0.0001	1.9	<0.0001	0.7	0.802
“Sweet”	15.9	<0.0001	5.2	0.006	15.2	<0.0001	2.5	<0.0001	1.6	<0.0001	0.6	0.953
Rancid	26.1	<0.0001	9.1	0.000	26.6	<0.0001	2.8	<0.0001	1.9	<0.0001	1.3	0.194
Cooked	31.1	<0.0001	2.0	0.139	1.5	0.142	1.9	0.001	1.7	<0.0001	0.8	0.706

Three-way analysis of variance (ANOVA) results illustrating panel performance for odor attribute intensities of green, “sweet”, rancid and cooked notes. Three independent variables were evaluated for a particular ingredient series ($pH_{process}$ 4) that was modified by temperature: 55, 75 and 95 °C ($T_{process}$) and treatment duration: 30 and 360 min ($t_{process}$) as triplicates: a) subject: representing the 21 panelists recruited; b) replicate: representing process as well as analytical replicates; and c) product type: representing each type of ingredient suspended at two application pH, either $pH_{utilization}$ 4 or 7.

elists, which should not hinder the interpretation. A detailed analysis of the results permitted to confirm this hypothesis. Therefore, all these performance results suggest that the panelists’ scoring was globally consistent (discriminative, repeatable and homogeneous), even if there was some inconsistency in the case of certain attributes, which was taken into account in the analysis of the results.

3.1.2. Difference of sensory description between ingredients from CATA test

All 36 attributes were ticked at least one time during the test. On average, 7 attributes per product were ticked by the judges to describe a sample. The least ticked attributes across all samples were petrol (1%), coffee (4%) and cigarette (4%), and the most ticked were hay (46%), mung bean (39%), potato (35%), lentil (30%) and cut grass (30%). Results showed significant differences of perception between the different ingredient suspensions for 28 out of 36 attributes (Cochran’s Q test ($p \leq 0.05$)). The significant attributes are presented on Table 3, along with the differences between ingredient suspensions with the post-hoc multiple pairwise comparison test by Critical difference (Sheskin) method. A comparison of the samples showed that the ingredients modified by process conditions and then suspended at two pH gave a diverse mix of odor complexities (Fig. 1). Specifically, a high variation between the ingredients at the different $pH_{utilization}$ was observed. Taking FBIC into account, attributes like cut grass, celery, fresh and spices were more frequently used at $pH_{utilization}$ 7 compared to $pH_{utilization}$ 4. Perception of rancid note was more frequently selected to describe the FBIC at $pH_{utilization}$ 4. Therefore, it seemed that the $pH_{utilization}$ had an impact on the release of different odor attributes for the same ingredient. The same effect was observed for the other ingredients modified by process conditions. The frequencies of odor attributes differed due to the $pH_{utilization}$ for the same modified concentrate. Also, there were differences between distinct modified concentrates, and between them and the FBIC. For instance, the attribute chocolate was not selected to describe the FBIC at $pH_{utilization}$ 7, but was selected as a descriptor for all the modified concentrates at this $pH_{utilization}$. Meanwhile, at $pH_{utilization}$ 4, the attribute was again absent for the FBIC and present for just a few modified concentrates (Table 3). The effect of process conditions, particularly the $pH_{utilization}$, in driving different odor perceptions was more clearly observed in the CA performed on significant attributes (Fig. 1). Here, the ingredient suspensions were divided primarily by the first dimension ($F1 = 36.56\%$), in which vinegar, meat, egg, chemical, rancid, burnt, citrus, red wine and spices were more pronounced in the $pH_{utilization}$ 4 suspensions, whereas chocolate, almond, hazelnut, banana, vanilla, coffee, caramel, fresh, milk, lentil, and wood attributes were featured mostly in $pH_{utilization}$ 7 suspensions (Fig. 1). The attributes smoky, cut grass, celery, earthy, mung bean were shared between the two clusters of $pH_{utilization}$. Separation of the ingredient suspensions by the second dimension ($F2 = 12.04\%$) was unclear and might have been caused by a combination of other process conditions ($pH_{process}$, $T_{process}$ and/or $t_{process}$). However, this separation was of lower importance compared to the impact of $pH_{utilization}$.

After this qualitative comparison between different ingredient suspensions, there was a need to describe their differences based on the intensities of odor notes. Four major odor attributes were quantified for all the samples by the panel.

3.1.3. Difference in note intensities between ingredients

For a clearer understanding of the effects of process conditions on different odors, the intensity profiling of the ingredients’ suspensions was performed based on the evaluation of key odors. A four-way ANOVA (Table 4) presented significant effects of all process conditions ($pH_{process}$, $T_{process}$, $t_{process}$ and $pH_{utilization}$) on green, “sweet”, rancid and cooked perceived intensities. Differences among groups were calculated by the Newman-Keuls post-hoc analysis. The FBIC concentrate had significantly higher green note and lower “sweet” note intensities compared to the modified ingredients grouped by different $pH_{process}$. It was also significantly lower in cooked notes as compared to the ingredients modified by $pH_{process}$ 2 and 11 and in rancid notes compared to the ingredients modified by $pH_{process}$ 2. The ingredients suspended at different $pH_{utilization}$ were significantly different in green, “sweet” and rancid notes ($p \leq 0.05$, Table 4). At $pH_{utilization}$ 7, the perceptions of green and “sweet” notes were perceived significantly higher compared to samples at $pH_{utilization}$ 4 ($p \leq 0.05$, Table 4). On the other hand, rancid intensity was perceived significantly higher in the samples at $pH_{utilization}$ 4 ($p \leq 0.05$, Table 4) than at $pH_{utilization}$ 7. It must be noted that while ANOVA showed significant differences in all odor intensities, the post-hoc analysis presented singular groups for $T_{process}$ and $t_{process}$ – suggesting the presence of high variations within the groups themselves.

The PCA biplot highlighted the description of the ingredient suspensions in regard to their respective perceived odor intensities. It explained 76% of the variances (Fig. 2). The biplot presented a predominant effect of the $pH_{utilization}$ in influencing the intensities of rancid and “sweet” notes. The “sweet” and rancid notes were associated away from each other, with rancid notes being perceived more intense at $pH_{utilization}$ 4 and “sweet” notes being perceived with higher intensity at $pH_{utilization}$ 7. Cooked and green notes were oppositely associated and were not in relation with the “sweet” and rancid notes. The exact reasoning for their opposition on the PCA biplot was not as clear as for the “sweet” and rancid notes (Fig. 2), compared to the significant differences seen between them from ANOVA (Table 4). Nevertheless, a notable impact of $pH_{utilization}$ 7 can be seen in the perception of cooked and green notes. For instance, the FBIC used at $pH_{utilization}$ 7 gave a significantly higher perception of green notes (Table 4) and is on the extreme end of the quadrant of rancid notes (Fig. 2). Additionally, the FBIC at $pH_{utilization}$ 7 was negatively associated with the cooked notes. Interestingly, for the same FBIC but at $pH_{utilization}$ 4 the green notes were not perceivable. The ingredients that gave higher perception of cooked notes were also mostly among the $pH_{utilization}$ 7 suspensions.

Table 3
Significant odor attributes from Check-All-That-Apply (CATA) odor sensory evaluation of fava bean ingredient suspensions.

	FBI	pH _{neutralization} 4															
		pH _{process} 2				pH _{process} 4				pH _{process} 6.4				pH _{process} 11			
		55 °C	75 °C	95 °C	55 °C	75 °C	95 °C	55 °C	75 °C	95 °C	55 °C	75 °C	95 °C	55 °C	75 °C	95 °C	
Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High		
Cut Grass	0.190 abc	0.333 abc	0.286 abc	0.333 abc	0.286 abc	0.238 abc	0.381 abc	0.333 abc	0.286 abc	0.333 abc	0.333 abc	0.238 abc	0.333 abc	0.286 abc	0.238 abc	0.238 abc	
Celery	0.238 ab	0.143 ab	0.238 abc	0.143 abc	0.238 abc	0.143 abc	0.095 abc	0.190 abc	0.238 abc	0.143 abc	0.095 abc	0.143 abc	0.095 abc	0.143 abc	0.238 abc	0.238 abc	
Leontide	0.333 ab	0.238 ab	0.190 abc	0.238 abc	0.143 abc	0.238 abc	0.338 abc	0.143 abc	0.338 abc	0.143 abc	0.238 abc	0.286 abc	0.143 abc	0.095 abc	0.238 abc	0.286 abc	
Mung Bean	0.429 a	0.333 a	0.286 a	0.429 a	0.476 a	0.476 a	0.381 a	0.429 a	0.381 a	0.429 a	0.333 a	0.429 a	0.524 a	0.381 a	0.429 a	0.333 a	
Fresh	0.238 ab	0.095 a	0.238 abc	0.143 abc	0.286 abc	0.048 abc	0.238 abc	0.190 abc	0.286 abc	0.238 abc	0.190 abc	0.286 abc	0.143 abc	0.190 abc	0.190 abc	0.286 abc	
Wood	0.143 ab	0.286 a	0.190 abc	0.143 abc	0.381 abc	0.286 abc	0.190 abc	0.238 abc	0.286 abc	0.333 abc	0.095 abc	0.143 abc	0.190 abc	0.238 abc	0.143 abc	0.238 abc	
Earthy	0.238 a	0.381 a	0.286 a	0.190 abc	0.238 abc	0.143 abc	0.095 abc	0.238 abc	0.143 abc	0.095 abc	0.190 abc	0.095 abc	0.143 abc	0.238 abc	0.143 abc	0.238 abc	
Spices	0.048 a	0.048 a	0.095 abc	0.048 abc	0.095 abc	0.190 abc	0.048 abc	0.048 abc	0.095 abc	0.143 abc	0.095 abc	0.143 abc	0.095 abc	0.190 abc	0.048 abc	0.143 abc	
Caramel	0 a	0.048 a	0.095 abc	0.048 abc	0.143 abc	0.048 abc	0.190 abc	0.048 abc	0.095 abc	0.048 abc	0.095 abc	0.143 abc	0.190 abc	0.048 abc	0.048 abc	0.143 abc	
Rancid	0.381 abcd	0.714 d	0.619 bcd	0.524 abc	0.429 abc	0.476 abc	0.381 abc	0.571 abc	0.714 abc	0.429 abc	0.619 abc	0.524 abc	0.429 abc	0.429 abc	0.571 abc	0.381 abc	
Burnt	0.143 ab	0.095 ab	0.095 abc	0.095 abc	0.095 abc	0.095 abc	0.095 abc	0.095 abc	0.048 abc	0.048 abc	0.095 abc	0.048 abc	0.095 abc	0.048 abc	0.095 abc	0.286 abc	
Smoky	0.190 ab	0.095 ab	0.143 abc	0.190 abc	0.048 abc	0.381 abc	0.048 abc	0.143 abc	0.095 abc	0.190 abc	0.190 abc	0.238 abc	0.190 abc	0.333 abc	0.190 abc	0.095 abc	
Coffee	0 a	0 a	0.048 abc	0.048 abc	0 a	0 a	0 a	0 a	0.048 abc	0 a	0 a	0 a	0 a	0.095 abc	0 a	0.048 abc	
Chocolate	0 a	0 a	0.048 abc	0.048 abc	0 a	0 a	0 a	0.048 abc	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0.048 abc	
Hazelnut	0.048 a	0.190 a	0.143 abc	0.286 abc	0.143 abc	0.095 abc	0.286 abc	0.095 abc	0.048 abc	0.238 abc	0.143 abc	0.143 abc	0.143 abc	0.048 abc	0.190 abc	0.048 abc	
Orange Blossom	0.095 abc	0 a	0.143 abc	0 a	0 a	0 a	0.048 abc	0.095 abc	0.048 abc	0.286 abc	0.095 abc	0.190 abc	0.048 abc	0 a	0 a	0.048 abc	
Vanilla	0.095 a	0.048 a	0.143 abc	0.095 abc	0.095 abc	0.143 abc	0.190 abc	0.095 abc	0.190 abc	0.095 abc	0.190 abc	0.095 abc	0.095 abc	0.190 abc	0.048 abc	0.143 abc	
Banana	0.190 a	0.048 a	0.095 abc	0.095 abc	0.143 abc	0.143 abc	0.095 abc	0.190 abc	0.143 abc	0.190 abc	0.333 abc	0.143 abc	0.095 abc	0.048 abc	0.048 abc	0.048 abc	
Almond	0.143 ab	0.381 ab	0.048 abc	0.286 abc	0.143 abc	0.143 abc	0.333 abc	0.238 abc	0.338 abc	0.190 abc	0.095 abc	0.143 abc	0.143 abc	0.143 abc	0.238 abc	0.095 abc	
Citrus	0.143 ab	0.143 ab	0.143 abc	0.095 abc	0.190 abc	0.238 abc	0.095 abc	0.143 abc	0.238 abc	0.333 abc	0.095 abc	0.143 abc	0.095 abc	0.190 abc	0.095 abc	0.238 abc	
Red Wine	0.048 ab	0 a	0.048 abc	0 a	0.143 abc	0.143 abc	0.048 abc	0.048 abc	0.095 abc	0.143 abc	0.143 abc	0.095 abc	0.143 abc	0.048 abc	0.048 abc	0.143 abc	
Vinegar	0.286 abc	0.190 abcd	0.286 abc	0.143 abc	0.143 abc	0.333 abc	0.190 abc	0.333 abc	0.286 abc	0.429 abc	0.333 abc	0.429 abc	0.333 abc	0.524 abc	0.571 abc	0.619 abc	
Milk	0.143 abc	0.143 abc	0.286 abc	0 a	0.143 abc	0.048 abc	0.238 abc	0.095 abc	0.143 abc	0.095 abc	0.048 abc	0.429 abc	0.238 abc	0.338 abc	0.095 abc	0.286 abc	
Egg	0.238 a	0.095 a	0.095 abc	0.190 abc	0.048 abc	0.095 abc	0.190 abc	0.048 abc	0.190 abc	0.048 abc	0.190 abc	0.095 abc	0.143 abc	0.095 abc	0.286 abc	0.238 abc	

(continued on next page)

Table 3 (continued)

FBIC	pH _{process 2}						pH _{process 4}						pH _{process 6.4}						pH _{process 11}						
	55 °C		75 °C		95 °C		55 °C		75 °C		95 °C		55 °C		75 °C		95 °C		55 °C		75 °C		95 °C		
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	
Vinegar	0.048	0	0.095	0	0.048	0	0.048	0.048	0	0.095	0	0.048	0.095	0.048	0.048	0.048	0.095	0.143	0	0.143	0.095	0.095	0.143	0.095	0.143
	ab	a	abc	a	ab	abcd	abcd	a	ab	abc	a	ab	abc	ab	ab	ab	abcd	a	a	abcd	abcd	a	abcd	abcd	abcd
Milk	0.095	0.048	0.095	0.238	0.190	0.143	0.286	0.190	0.381	0.238	0.095	0.476	0.190	0.286	0.190	0.429	0.095	0.143	0.095	0.190	0.190	0.143	0.048	0.048	0.048
	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Egg	0.095	0.143	0.095	0.048	0.095	0.143	0.095	0.095	0.048	0.095	0	0.095	0	0.048	0	0	0.095	0.048	0.048	0.048	0.048	0.095	0.143	0.095	0.143
	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Meat	0	0.143	0.143	0.048	0	0.095	0.190	0	0	0.048	0.095	0	0.048	0	0	0.048	0	0.048	0.048	0.048	0.048	0	0.143	0.048	0.143
	a	ab	ab	a	a	ab	a	a	a	ab	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ammoniac	0	0.143	0.095	0.190	0.143	0.095	0.143	0.095	0.048	0.095	0.095	0	0	0.095	0.048	0.095	0.095	0.095	0.143	0.143	0.143	0.190	0.190	0.190	0.190
	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Chemical	0.095	0.095	0.143	0.095	0.143	0.095	0.190	0.048	0.048	0	0.095	0	0.048	0	0.143	0.095	0.048	0.095	0.048	0.048	0.048	0.048	0.048	0.048	0.095
	ab	ab	ab	ab	ab	ab	ab	ab	a	a	a	a	a	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab

Multiple pairwise comparison Critical difference / Shekkin procedure of significant odor attributes for the suspensions of fava Bean initial concentrate (FBIC) and the same modified by pH: 2, 4, 6.4 and 11 (pH_{process}), temperature: 55, 75 and 95 °C (T_{process}) and treatment duration: 30 and 360 min (t_{process}), and then utilized at application pH: 4 and 7 (pH_{utilization}).



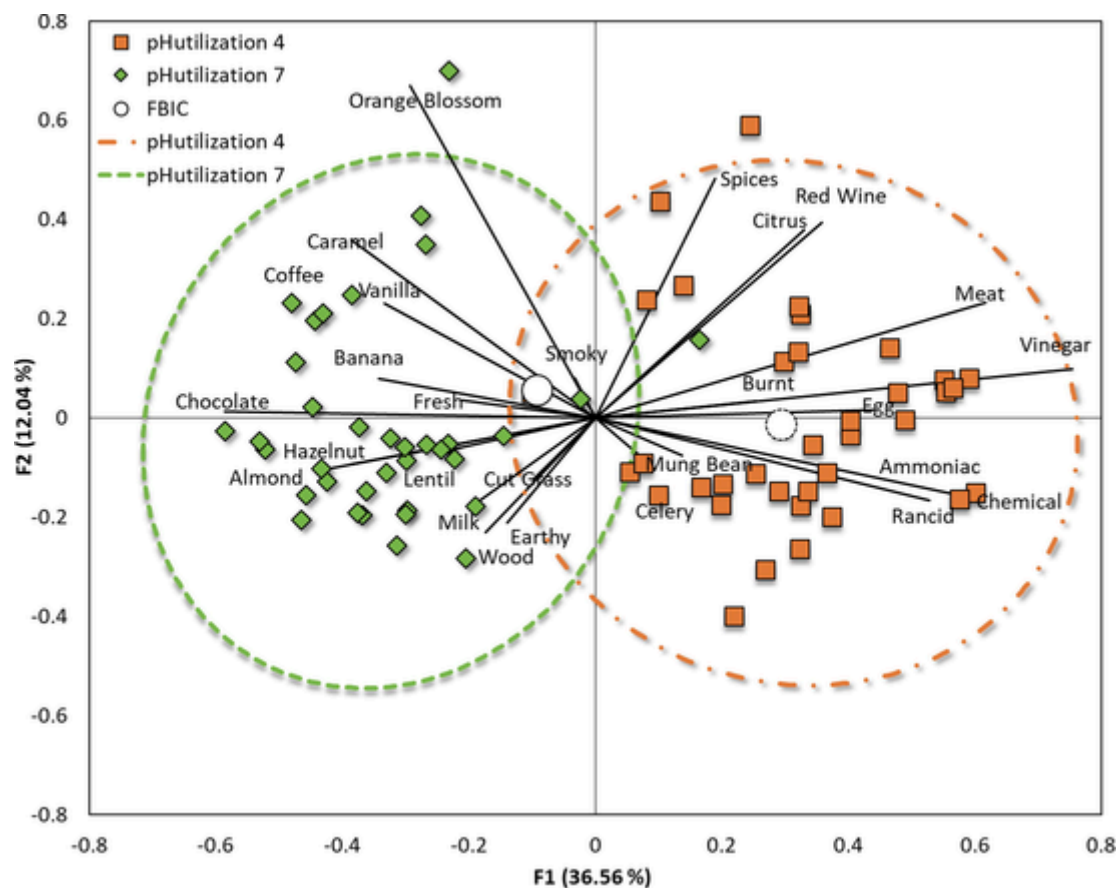


Fig. 1. Odor attributes of fava concentrate suspensions. Correspondence analysis (CA) of the data projecting different ingredient aqueous suspensions as points and significant odor attributes as lines on the biplot plane. The ingredients studied are the FBIC (fava bean initial concentrate, circular points) and the same modified by pH: 2, 4, 6.4 and 11 ($pH_{process}$), temperature: 55, 75 and 95 °C ($T_{process}$) and treatment duration: 30 and 360 min ($t_{process}$), and then utilized at application pH: 4 and 7 ($pH_{utilization}$). CA was performed on the binary data (no pre-processing) from check-all-that-apply (CATA) sensory analysis. Confidence ellipses ($\alpha = 0.05$) were constructed on the sample coordinates grouped by the $pH_{utilization}$. The orange-square points or dash-dot line and the green-diamond points or dash line respectively represent $pH_{utilization}$ 4 and 7 data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Headspace volatile analysis

The volatile compounds present in the headspace of the ingredient suspensions were extracted and analyzed by HS-SPME-GC-MS. Eighty-three volatile compounds were identified in the various ingredients suspended at $pH_{utilization}$ 4 and 7, including 11 alkanes, 4 alkenes, 13 alcohols, 25 aldehydes, 12 ketones, 2 esters, 3 organic acids, 2 aromatic hydrocarbons, 6 furanoids and 5 pyranoids (Table 5).

The volatile compounds detected were further grouped by chemical classes, and their relative cumulative areas provided a better understanding of the chemistry of ingredients with respect to process conditions (Fig. 3). The following results present the highlights from the generation and release of headspace volatiles due to process conditions. Their role in odor activity is discussed in subsequent sections and illustrations (Fig. 5).

Firstly, as indicated by the results from the odor sensory profiling of the FBIC suspension, the headspace volatile chemistry also revealed differences between the two $pH_{utilization}$. Overall, aldehydes and alcohols were the most abundant compounds detected for the FBIC at both $pH_{utilization}$ (Fig. 3; Table 5), even if they are not the only notable odor compounds of the samples. For the FBIC at $pH_{utilization}$ 4, the detected signals of aldehydes were the highest, i.e. 76% of the total chromatogram peak area compared to 10% for FBIC at $pH_{utilization}$ 7. Conversely, at $pH_{utilization}$ 7, alcohol signals (instead of aldehydes) were predominantly detected, contributing to 72% of the total peak area vs 13% at $pH_{utilization}$ 4. Amongst these volatile groups, hexanal and 1-hexanol were examined in greater depth owing to their predominance in peak

area signals within their respective chemical families (Table 5). Hexanal gave the highest normalized signal (372.8 area/ area_{d7-heptanol}/ g d.b.) amongst all the detected volatiles at $pH_{utilization}$ 4, whereas at $pH_{utilization}$ 7, 1-hexanol gave the highest signal (277.1 area/ area_{d7-heptanol}/ g d.b.). Hexanal drastically decreased when the FBIC was suspended at $pH_{utilization}$ 7 (30.3 area/ area_{d7-heptanol}/ g d.b.) compared to $pH_{utilization}$ 4. On the other hand, 1-hexanol was lower at $pH_{utilization}$ 4 (51.1 area/ area_{d7-heptanol}/ g d.b.) compared to $pH_{utilization}$ 7. In regard to other individual molecules, there was also a qualitative difference in the volatile profiles observed. A total of 32 volatile compounds were detected in the FBIC at $pH_{utilization}$ 7. In addition to these, 26 more compounds were detected at $pH_{utilization}$ 4, including aldehydes (butanal, heptanal, octanal, nonanal, decanal, 2-methylpropanal, 3-methylbutanal, (*E*)-2-pentenal, 2-hexenal, (*E,E*)-2,4-heptanedienal, 2-nonenal, 2,4-nonadienal, 2,4-decadienal, 2-undecenal, 9-octadecanal), alcohols (2-ethyl-1-hexanol, (*Z,Z*)-4,5-dimethyl-2-hepten-3-ol, 2-phenylethanol), furanoids (2-butylfuran, (*Z*)-2-(2-pentenyl)furan), one alkane (tetradecane), one alkene (1-heptene), ketones (2-heptanone, 3-octen-2-one) and organic acids (octanoic acid, nonanoic acid). The effect of $pH_{utilization}$ was also observed after ingredient modification, but less pronounced as in the case of the FBIC. With the exception of furfural, 3-methylbutylacetate, 2-ethyl-1-hexanol and 9-octadecanal, the rest of the volatile compounds were significantly different between $pH_{utilization}$ 4 and 7 ($p \leq 0.05$) for all the ingredients. Remarkably, the impact of $pH_{utilization}$ on the detection levels of aldehydes and alcohols was not as pronounced in the modified ingredients, suggesting a reduced impact of the change in matrix on the relative release of volatile

Table 4

Difference in intensities of different odor notes during sensory evaluation of fava bean ingredient suspensions.

Effects		Green	“Sweet”	Rancid	Cooked
pH _{process}	FBIC	6.155 a	2.914b	3.134b	3.179b
	pH _{process} 2	4.895b	4.202 a	4.257 a	4.326 a
	pH _{process} 4	4.662b	4.687 a	3.301b	3.722 ab
	pH _{process} 6.4	4.744b	4.638 a	3.293b	3.774 ab
	pH _{process} 11	4.558b	4.071 a	3.309b	4.349 a
	<i>p-Value</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001
T _{process}	FBIC	4.953 a	4.317 a	3.466 a	3.926 a
	55 °C	5.118 a	3.801 a	3.351 a	3.874 a
	75 °C	4.987 a	3.975 a	3.553 a	3.755 a
	95 °C	4.953 a	4.317 a	3.466 a	3.926 a
		<i>p-Value</i>	< 0.0001	< 0.0001	< 0.0001
t _{process}	FBIC	5.017 a	4.046 a	3.461 a	3.802 a
	Low	5.017 a	4.046 a	3.461 a	3.802 a
	High	4.976 a	4.215 a	3.454 a	4.007 a
		<i>p-Value</i>	< 0.0001	< 0.0001	< 0.0001
pH _{utilization}	pH _{utilization} 4	4.726b	3.498b	4.470 a	3.801 a
	pH _{utilization} 7	5.280 a	4.707 a	2.448b	3.940 a
		<i>p-Value</i>	< 0.0001	< 0.0001	< 0.0001

Four-way analysis of variance (ANOVA) ($\alpha = 0.05$), followed by the Newman-Keuls post-hoc analysis illustrating the means of different note intensities across samples and panelists that varied as a function of different process conditions (effects). The ingredients studied are fava bean initial concentrate (FBIC) and the same modified by pH: 2, 4, 6.4 and 11 (pH_{process}), temperature: 55, 75 and 95 °C (T_{process}) and treatment duration: 30 and 360 min (t_{process}) and then utilized at application pH: 4 and 7 (pH_{utilization}).

classes – as in the case of mildly processed ingredients such as the fava bean air-classified concentrate (Fig. 3). All this data suggested that the two pH_{utilization} might have led to changes in the ingredient suspension matrices, enhancing or hampering the release of volatiles in the head-space.

Apart from the effect of pH_{utilization}, there were differences between the ingredients by virtue of the process conditions (pH_{process}, T_{process} and t_{process}) used for their modification (Fig. 3).

Differences in the proportions of volatile class signals also showed effects of process conditions during ingredient modification, especially for pH_{process}. Regarding the different volatile groups detected in different series of ingredients modified by pH_{process}, aldehyde signals were the highest in proportion amongst all the ingredients, independently of the pH_{process} series. Interestingly, differences based on pH_{process} were present for the volatile groups having secondary relative contribution, i.e. furanoids, terpenoids, alcohols and ketones signals at pH_{process} 2, 4, 6.4 and 11 series, respectively (Fig. 3). Looking closely into the different individual molecules, there were qualitative and quantitative differences found between the different modified ingredients (Table 5). To compare different ingredients by their pH_{process}, 86, 87, 72 and 78 volatiles were respectively detected in pH_{process} 2, 4, 6.4 and 11 series at pH_{utilization} 4 vs, respectively, 88, 80, 49 and 67 volatiles at pH_{utilization} 7 (Table 5). All volatiles, except furfural, 3-methylbutylacetate, 2-ethyl-1-hexanol and 9-octadecanal, were significantly different within the different groups of ingredients processed by pH_{process}, T_{process} and t_{process} ($p \leq 0.05$). Comparing volatile generation by different pH_{process} series, the non-pH adjusted process generated the least amount of volatiles compared to the pH-modified processes during the modification step. This suggested that pH_{process} may have driven changes in flavor through possibly different reactions. Furthermore, the impact of T_{process} and t_{process} was significant in determining to what extent these volatile generating reactions could occur.

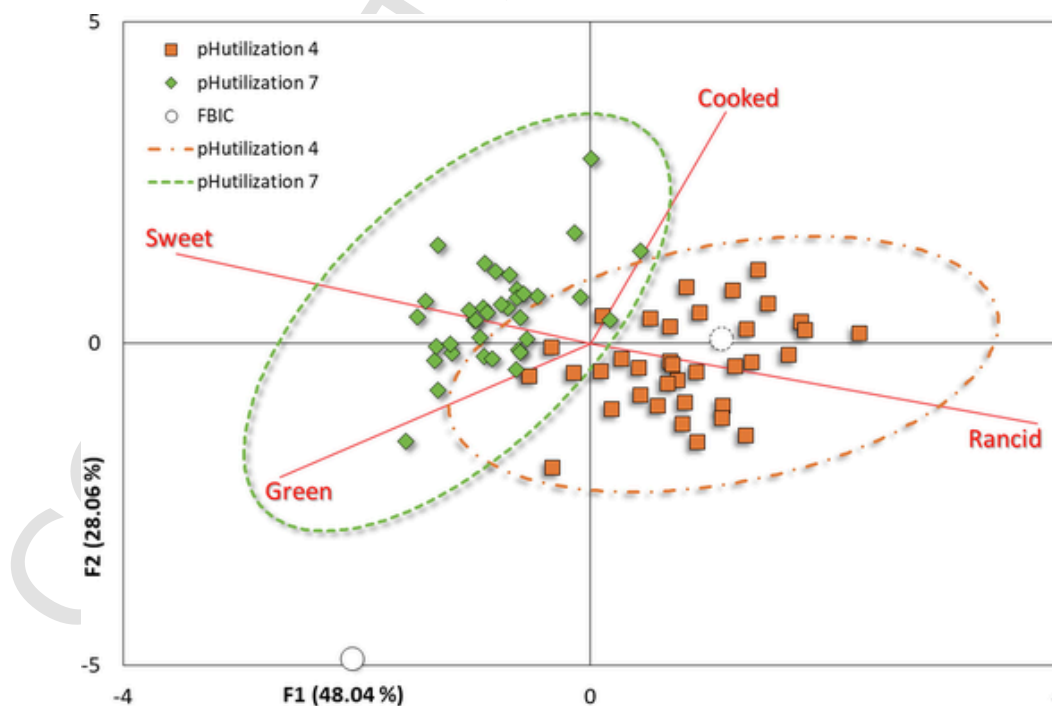


Fig. 2. Odor intensities of fava bean concentrate suspensions. Principal Component Analysis (PCA) projections of the different ingredient aqueous suspensions as points and their odor note intensities as lines on the plane. The ingredients studied are the FBIC (fava bean initial concentrate, circular points) and the same modified by pH: 2, 4, 6.4 and 11 (pH_{process}), temperature: 55, 75 and 95 °C (T_{process}) and treatment duration: 30 and 360 min (t_{process}), and then utilized at application pH: 4 and 7 (pH_{utilization}). The PCA analysis was performed on the odor intensity scores (no pre-processing) obtained from the sensory data. Confidence ellipses ($\alpha = 0.05$) were constructed on the sample coordinates grouped by the pH_{utilization}. The orange-square points or dash-dot line and the green-diamond points or dash line respectively represent pH_{utilization} 4 and 7 data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 5
Volatile compounds extracted from fava bean ingredient suspensions.

Chemical Group	Compound	CAS Number	Experimental Retention Index	Reference Retention Index ¹	Theoretical Odor Descriptor ^{2,3}	Extracted Ions used for Semi-Quantification	
Alkanes	Pentane	109-66-0	<718	NA	Alkane	41, 43, 57, 72	
	Heptane	142-82-5	<718	NA	alkane, ethereal, sweet	57, 71, 100	
	4-Methyloctane	2216-34-4	857	864	na	85, 98, 128	
	3-Methyl-6-Methyleneoctane	74630-07-2	937	NA	na	55, 69, 70, 71, 83, 84, 112, 140	
	2,7-Dimethyloctane	1072-16-8	984	930	na	56, 57, 71, 85, 86, 99, 100	
	2,2,4,6,6-Pentamethylheptane	13475-82-6	985	997	na	56, 57, 71, 85, 99, 112, 113, 155	
	Decane	124-18-5	998	1000	Alkane	57, 71, 85, 98, 99, 114	
	2,2,4,4-Tetramethyloctane	62183-79-3	1021	NA	na	57, 99, 113	
	Undecane	1120-21-4	1097	1100	Alkane	56, 57, 70, 71, 85, 99, 113, 127, 156	
	Dodecane	112-40-3	1199	1200	Alkane	55, 57, 71, 85, 99, 112, 127, 1170	
	Tetradecane	629-59-4	1399	1400	Alkane, waxy, mild	57, 71, 85, 99, 198	
	Alkenes	1-Heptene	592-76-7	<718	689	na	55, 56, 69, 70, 98
		1-Octene	111-66-0	786	789	Gasoline	55, 56, 70, 83, 84, 112
2,4-Dimethyl-1-Heptene		19549-87-2	833	855	na	55, 57, 69, 70, 83, 126	
4-Cyanocyclohexene		100-45-8	1011	na	na	54, 67, 79, 80, 106,107	
Alcohols	1-Penten-3-ol	616-25-1	<718	688	butter, pungent, tropical, horseradish, green, vegetable, bitter, fruity	57, 71, 86	
	(Z)-3-Methylcyclohexanol	5454-79-5	<718	na	na	57, 71, 81, 96	
	(E)-3-Methylcyclohexanol	7443-55-2	<718	na	na	57, 71, 81, 96, 97, 112	
	3-Methyl-1-Butanol	123-51-3	727	737	oil, alcoholic, burnt, whiskey, malt, banana, fusel, fruity	45, 55, 57, 70	
	1-Pentanol	71-41-0	759	764	oil, balsamic, vanilla, fusel, sweet, balsam	55, 56, 57, 70	
	3-Hexen-1-ol	544-12-7	848	858	moss, bitter, fresh, fatty, earthy, intensely, green	55, 67, 82,100	
	1-Hexanol	111-27-3	866	869	oil, alcoholic, ethereal, resin, fusel, sweet, fruity, flower, green	45, 55, 56, 57, 69, 84	
	1-Heptanol	111-70-6	968	970	leafy, coconut, herbal, peony, strawberry, chemical, musty, sweet, woody, violet, green	55, 56, 57, 68, 69, 70, 83, 98	
	1-Octan-3-ol	589-98-0	978	992	citrus, nut, moss, herbal, earthy, woody, melon, minty, mushroom, spicy	55, 59, 83, 101, 112	
	2-Ethyl-1-Hexanol	104-76-7	1026	1029	citrus, rose, fresh, floral, oily, sweet, green	57, 70, 83, 84, 98, 112, 130	
	1-Octanol	111-87-5	1068	1079	burnt, orange, rose, waxy, chemical, metal, aldehydic, mushroom, green	55, 56, 69, 70, 84, 97, 98, 130	
	(Z,Z)-4,5-Dimethyl-2-Hepten-3-ol	55956-37-1	1091	na	na	55, 71, 72, 100, 109, 124, 142	
	Linalool	78-70-6	1096	1102	lemon, citrus, orange, floral, sweet, woody, blueberry, bois de rose, lavender, flower, green	55, 67, 69, 71, 80, 83, 93, 94, 121, 136, 139	
Aldehydes	Acetaldehyde	75-07-0	<718	714	ethereal, ether, whiskey, pungent, fruity, aldehydic	43, 44	
	2-Methylpropanal	78-84-2	<718	550	malt, fresh, floral, pungent, aldehydic, green	41, 43, 72	
	Butanal	123-72-8	<718	601	bready, chocolate, creamy, pear, vegetable, fatty, earthy, woody, pungent, nutty, wine like, meaty, spicy, citrus, herbaceous, green, apple, floral, musty, cocoa, fruity, malty	44, 57, 72	
	3-Methylbutanal	590-86-3	<718	655	peach, sour, chocolate, ethereal, malt, fatty, aldehydic	44, 57, 58, 71, 86	
	2-Methylbutanal	96-17-3	<718	661	musty, coffee, cocoa, nutty, almond	57, 58, 71, 86	
	Pentanal	110-62-3	<718	696	bready, fermented, berry, malt, pungent, fruity, nutty, almond	44, 58, 71, 86	
	(E)-2-Pentenal	764-39-6	745	735	apple, orange, tomato, strawberry, fruit, pungent, fruity, green	55, 70, 91, 106	
	3-Methylhexanal	19269-28-4	783	na	sweet green	55, 57, 70, 81, 86, 114	
	Hexanal	66-25-1	800	812	leafy, grass, sweaty, tallow, fat, fresh, fatty, fruity, aldehydic, green	44, 55, 56, 57, 67, 72, 82	
	2-Hexenal	505-57-7	845	854	leafy, apple, cheesy, vegetable, fat, banana, rancid, fatty, sweet, plum, fruity, aldehydic, almond, green	55, 69, 83,98	
	Heptanal	111-71-7	901	914	citrus, ozone, fat, herbal, fresh, wine-lee, rancid, fatty, aldehydic, green	44, 45, 55, 57, 68, 70, 71, 81, 86, 96	

(continued on next page)

Table 5 (continued)

Chemical Group	Compound	CAS Number	Experimental Retention Index	Reference Retention Index ¹	Theoretical Odor Descriptor ^{2,3}	Extracted Ions used for Semi-Quantification
	(Z)-2-Heptenal	57266-86-1	952	957	na	55, 70, 83, 97, 112
	(E,E)-2,4-Heptanedienal	4313-03-5	993	992	hazelnut, cinnamon	53, 67, 81, 82, 95, 110
	Octanal	124-13-0	1001	1005	lemon, citrus, soap, orange peel, fat, waxy, fatty, aldehydic, green	57, 69, 84, 85, 100, 128
	2-Octenal	2363-89-5	1053	1060	nut, fat, herbal, fresh, green, fatty, banana, waxy, leaf, cucumber	55, 57, 69, 70, 83, 97, 98, 111, 126
	4-Nonenal	2277-16-9	1094	1096	fruity	54, 55, 67, 83, 84, 96, 98, 122, 140
	Nonanal	124-19-6	1102	1102	citrus, lime, orange peel, rose, fat, green, fishy, waxy, fresh, fatty, peely, aldehydic, orris, grapefruit	44, 45, 54, 55, 56, 57, 67, 70, 82, 98, 114, 124
	2-Dodecenal	4826-62-4	1111	na	citrus, orange, fat, herbal, waxy, mandarin, sweet, fruity, aldehydic, metallic, green	55, 69, 70, 83, 84, 97, 98, 111, 138, 164, 182
	2-Nonenal	18829-56-6	1160	1162	waxy, green, fatty, paper, melon, cucumber	55, 70, 83, 84, 96, 111, 122, 140
	Decanal	112-31-2	1203	1208	citrus, soap, orange peel, tallow, waxy, floral, sweet, aldehydic	55, 57, 70, 71, 82, 95, 112, 128, 138
	2,4-Nonadienal	5910-87-2	1214	1214	fatty, melon, waxy, green, violet leaf, cucumber, tropical fruit, chicken fat	53, 67, 81, 82, 95, 109, 138
	2,4-Decadienal	2363-88-4	1306	1284	citrus, orange, nut, wax, meat, fat, fresh, fatty, oily, cucumber, sweet, melon, pumpkin, fried, green	55, 67, 81, 83, 95, 96, 123, 152
	2-Undecenal	2463-77-6	1361	1355	citrus, soap, orange peel, fat, fresh, sweet, fruity, green	54, 57, 69, 70, 82, 83, 84, 97, 98, 111, 124, 150, 168
	2-Butyl-2-Octenal	13019-16-4	1369	1389	aldehydic, green, watery, fruity, metallic, oily, tropical, fatty, sweaty, goaty	55, 69, 83, 95, 111, 125, 1182
	9-Octadecenal	5090-41-5	1904	na	fatty	55, 69, 81, 83, 98, 111, 121, 135, 152, 248, 266
Ketones	2-Butanone	78-93-3	<718	622	ethereal, ether, fruity, acetone, camphor	57, 72
	2-Pentanone	107-87-9	<718	693	potato, alcohol, ether, ethereal, wine, banana, fishy, fruit, sweet, woody, fruity	58, 70, 71, 86
	3-Penten-2-one	3102-33-8	727	721	acetone, fishy, fruity, phenolic	69, 70, 84
	2-Heptanone	110-43-0	887	900	coconut, soap, herbal, sweet, woody, fruity, spicy, cinnamon	58, 71, 99, 114
	1-Octen-3-one	4312-99-6	973	978	herbal, earthy, metal, musty, mushroom, dirty	55, 70, 83, 97, 111, 126
	6-Methyl-5-Hepten-2-one	110-93-0	981	995	pepper, apple, mushroom, citrus, musty, rubber, nutty, green, hazelnut, bitter, lemongrass	55, 69, 93, 108, 126
	3-Octen-2-one	1669-44-9	1033	1040	crushed bug, nut, herbal, earthy, hay, sweet, blueberry, mushroom, spicy	55, 69, 83, 97, 111, 126
	3,5-Octadien-2-one	38284-27-4	1064	1098	fat, fatty, fruit, fruity, grassy, mushroom, green	53, 55, 79, 81, 95, 109, 124
	3-Nonanone	925-78-0	1082	1082	jasmin, herbal, fresh, sweet, fruity, leaf, spicy	55, 57, 58, 72, 85, 95, 99, 113, 114, 142
	2-Nonanone	821-55-6	1087	1096	soap, herbal, fresh, fishy, hot milk, earthy, sweet, soapy, weedy, green	57, 58, 59, 71, 85, 99, 127, 142
	3-Undecanone	2216-87-7	1284	1283	na	57, 72, 73, 85, 96, 123, 1170
	2-Undecanone	112-12-9	1287	1291	orange, waxy, fresh, fatty, floral, iris, creamy, fruity, orris, green	58, 71, 85, 96, 112, 110, 155, 170, 171
	Esters	3-Methylbutylacetate	123-92-2	873	878	bitter, solvent, banana, fruity, sweet
(Z)-3-Octenylacetate		69668-83-3	1072	na	na	54, 68, 69, 81, 82, 95, 110, 111
Acids	Acetic Acid	64-19-7	<718	646	sour, pungent, sharp, vinegar	45, 60
	Octanoic Acid	106-32-1	1197	1194	Cheesy, sweat, vegetable, waxy, fatty, rancid, oily, cheese	57, 60, 70, 88, 101, 127, 129, 172
	Nonanoic Acid	112-05-0	1269	1272	cultured dairy, fat, waxy, green, dirty, cheese	57, 60, 73, 83, 98, 115, 129, 1158
Aromatic Hydrocarbons	2-Phenylethanol	60-12-8	1104	1088	lilac, rose, rose water, honey, rose flower, floral, spice, bitter, rose dried	51, 65, 77, 91, 92, 93, 122
	4-Propylbenzaldehyde	28785-06-0	1272	na	na	51, 65, 89, 91, 92, 105, 119, 120, 147, 148
Furanoids	2-Methylfuran	534-22-5	<718	613	ethereal, acetone, chocolate	53, 81, 82
	Furfural	98-01-1	826	830	fragrant, bread, woody, sweet, baked, almond	67,96

(continued on next page)

Table 5 (continued)

Chemical Group	Compound	CAS Number	Experimental Retention Index	Reference Retention Index ¹	Theoretical Odor Descriptor ^{2,3}	Extracted Ions used for Semi-Quantification
	2-Butylfuran	4466-24-4	886	893	mild, wine, sweet, fruity, spicy	53,67,81,82,95,96,124
	2-Pentylfuran	3777-69-3	987	993	butter, green bean, vegetable, earthy, beany, fruity, metallic, green	53, 81, 82, 95, 138
	(Z)-2-(2-Pentenyl)furan	70424-13-4	996	1003	na	53,79,94,107,136
	5-Heptyldihydro-2(3H)-furanone	104-67-6	1357	na	peach, coconut, lactonic, apricot, fatty, creamy, fruity, ketonic	55, 56, 73, 85, 100, 128, 129, 166
Terpenoids	1-R- α -Pinene	7785-70-8	926	937	harsh, turpentine, minty, fresh, earthy, sweet, woody, terpene, pine, camphor	77, 79, 91, 92, 93, 105, 121, 136
	3-Carene	13466-78-9	1003	1009	lemon, citrus, sweet, resin	65,67,79,91,93,105,121, 136
	D-Limonene	138-86-3	1023	1029	mint, lemon, citrus, orange, fresh, sweet	53, 67, 68, 79, 93, 107, 121, 136
	(+)- α -Terpineol	7785-53-7	1193	1190	oil, mint, citrus, lilac, floral, woody, anise, terpene, pine	55, 59, 67, 81, 93, 95, 121, 136, 115
	(Z)- β -Farnesene	28973-97-9	1499	na	wood, citrus, sweet, woody	55, 67, 69, 81, 93, 105, 120, 133, 148, 161, 162, 189, 204
Deuterated Standard	D7-heptanol	1219804-99-5	964	-	-	45, 62,77

Detection of volatile compounds extracted from the headspace of different ingredients suspensions by automated solid-phase micro-extraction (HS-SPME) and then analyzed by gas chromatography coupled with mass spectroscopy (GC-MS). The ingredients in study are the FBIC (fava bean initial concentrate) and the same modified by pH: 2, 4, 6.4 and 11 ($\text{pH}_{\text{process}}$), temperature: 55, 75 and 95 °C (T_{process}) and treatment duration: 30 and 360 min (t_{process}) and then utilized at application pH: 4 and 7 ($\text{pH}_{\text{utilization}}$).

¹ = National Institute of Standards and Technology (NIST), 2021.

² = Arn & Acree, 1998.

³ = Garg et al., 2017.

na = not available

Thus, in a nutshell, two main effects were clear: 1) effect of $\text{pH}_{\text{utilization}}$, which was strikingly noticeable for the FBIC and less strong but still significant for all the modified ingredients, affecting the release of volatile compounds; and 2) effect of $\text{pH}_{\text{process}}$, which was evident while looking into the relative proportions of the different volatile groups and led to changes in chemical complexity. Despite these results, due to the heavy influence of the matrix in the release of volatiles, it was still unclear as to how T_{process} and t_{process} affect volatile complexity. As these conditions were statistically proven to be significant in influencing the volatile change, PCA was used to have a better overview of the volatile complexity as a function of process effects. Since the $\text{pH}_{\text{utilization}}$ was shown to influence the volatile release, the individual PCA plots for $\text{pH}_{\text{utilization}}$ 4 (Fig. 4A, 56% explained variance) and $\text{pH}_{\text{utilization}}$ 7 (Fig. 4B, 54% explained variance) were chosen for a better understanding. In both plots, the different ingredient suspensions (colored points on the plot) were separated based on their $\text{pH}_{\text{process}}$ during modification. Certain ingredients ($\text{pH}_{\text{process}}$ 2) were positively associated with the variation of large number of volatiles in the plots (Fig. 4A and B). Furthermore, all the clusters were associated with the two different $\text{pH}_{\text{utilization}}$. For instance, the FBIC at $\text{pH}_{\text{utilization}}$ 4 was closer to the cluster of $\text{pH}_{\text{process}}$ 11 ingredients and positively associated with the release of volatiles including acetaldehyde, 3-penten-2-one, 2-ethyl-1-hexanol, 3-carene and octanoic acid (examples from different volatile groups) (Fig. 4A). It was negatively associated with the release of volatiles such as undecane, 1-heptanol, (+)- α -terpineol, 3-nonanone and acetic acid. The same FBIC was further away from the $\text{pH}_{\text{process}}$ 11 cluster, and distinctly separated from the rest of the modified ingredients at $\text{pH}_{\text{utilization}}$ 7 (Fig. 4B). From these PCA plots, it was also possible to extract information about the effects of T_{process} and t_{process} . Firstly, the extent of the effect of T_{process} and t_{process} depended greatly on the type of $\text{pH}_{\text{utilization}}$ and $\text{pH}_{\text{process}}$. The ingredients from $\text{pH}_{\text{process}}$ 2 series in particular showed a great separation along both F1 and F2 axes, due to the effects of T_{process} and t_{process} at both $\text{pH}_{\text{utilization}}$. Variations of T_{process} and t_{process} were less pronounced for $\text{pH}_{\text{process}}$ 6.4 and 11 series as they lied only along F2 axes that accounted for 12–15% of variances explained in

both the PCA plots. Effects of T_{process} and t_{process} were the least impactful for $\text{pH}_{\text{process}}$ 4 series (Fig. 4A and B).

4. Discussion

Odor description and quantification, and volatile compounds' analysis were evaluated together using MFA (Fig. 5) in order to estimate resemblances and discrepancies between odor perception and chemical composition (Abdel-Kader, 2000). In this association between odor perception and volatile chemistry, only those volatiles with theoretical odor descriptors have been selected (Table 5). Through this, an attempt to simplify this complexity was made first by looking into the odor intensities that divided the quadrants into four separate perceptive zones. The first was a more "sweet", less rancid perceptive zone, specifically defined by three clusters viz. a) banana, almond, lentil and vanilla notes; b) hazelnut, chocolate and caramel notes; and c) coffee, earthy and woody notes. The second was a more cooked, less green zone of perception, specified by a) smoky and burnt; b) red wine; as well as c) ammoniac and meat note clusters. The third zone represented a more green, less cooked perception and was characterized by a) milk, orange blossom; b) fresh; and c) cut grass note clusters. Finally, the last quadrant was a more rancid, less sweet perceptive zone, associated with a) spices; b) egg, vinegar, chemical; c) rancid; and d) citrus, mung bean and celery note clusters (Fig. 5).

MFA analysis contributed to the understanding of odor perception from the composition in flavor compounds and the impact of process conditions on both composition and perception. The first dimension was characterized by $\text{pH}_{\text{process}}$, T_{process} and t_{process} , thus presenting the degree of ingredient processing, whereas the second represented more the conditions during food application, as they were separated by the $\text{pH}_{\text{utilization}}$ (Fig. 5). The process conditions used for fava bean ingredient modification were either gentler ($\text{pH}_{\text{process}} = 4$ and 6.4, $T_{\text{process}} = 55$ °C, $t_{\text{process}} = \text{Low}$) or more vigorous ($\text{pH}_{\text{process}} = 2$ or 11, $T_{\text{process}} > 55$ °C, $t_{\text{process}} = \text{High}$), and this division could be seen clearly from the biplot. The FBIC (unmodified air classified concentrate) also

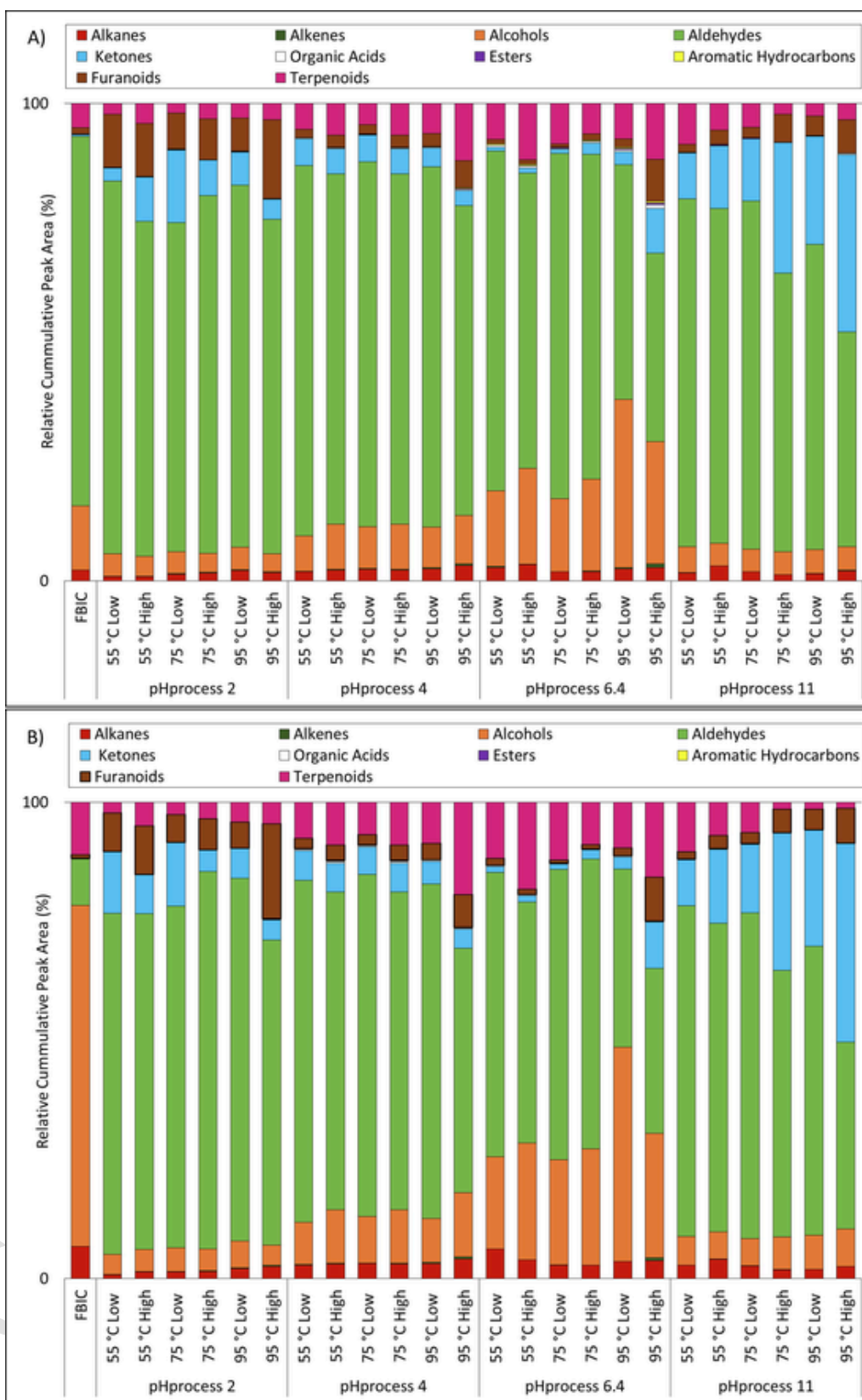


Fig. 3. Volatiles families detected for fava bean concentrate suspensions. Cumulative relative peak areas of the different chemical classes of volatile compounds extracted from the headspace of different ingredients suspensions by automated solid-phase micro-extraction (HS-SPME) and analyzed by gas chromatography coupled with mass spectroscopy (GC-MS). The ingredients studied are the FBIC (fava bean initial concentrate, circular points) and the same modified by pH: 2, 4, 6.4

and 11 ($\text{pH}_{\text{process}}$), temperature: 55, 75 and 95 °C (T_{process}) and treatment duration: 30 and 360 min (t_{process}), and then utilized at application pH: 4 and 7 ($\text{pH}_{\text{utilization}}$).

lied in the quadrant of gentle processing, thus making this distinction further apparent.

4.1. Effect of degree of ingredient processing

As illustrated by the results, the degree of ingredient processing clearly impacts their odor perception and volatile composition. Gentler processing, including the presence of the FBIC, was associated to a more green perception, with the FBIC being closely related to cut grass notes (Fig. 5). Perceptions of “green”, “beany”, “raw” and “fresh” notes have often been associated with raw legumes including beans, pea, lupin and soy (MacLeod et al., 1988; Reineccius & Heath, 2006). The FBIC used in this study was obtained by milling of dried and dehulled beans, followed by air classification. Although certain volatile compounds can occur naturally, the majority of them are formed by processing (Chambers, 2019; Sharan, Zanghelini, et al., 2021). The series of reactions that enable formation of volatiles span from the harvesting to storage, and then to bean processing. Bean dehulling and milling results in breakdown of tissues and exposure of flavor precursors (e.g. lipids, proteins and sugars) to active enzymes (e.g. lipases, lipoxygenases, dehydrogenases), thus enabling substrate degradation into flavor molecules (Siegmund, 2015; Roland et al., 2017; Sharan, Zanghelini, et al., 2021). For pulses in general, grassy notes have often been reported owing to higher alcohols and aldehydes levels (Mishra et al., 2017; Ong & Liu, 2018; Sharan, Zanghelini, et al., 2021). This is in accordance with the higher levels of aldehydes and alcohols found for the FBIC in this study (Fig. 3). Higher levels of aldehydes and alcohols, as well as certain furans and ketones, have often been associated with the oxidation of polyunsaturated fatty acids (PUFA), i.e. linoleic and linolenic acids in pulses, leading to the formation of compounds associated with grassy, beany and green attributes (Rackis, Sessa, & Honig, 1979; MacLeod et al., 1988; Reineccius & Heath, 2006; Roland et al., 2017). Lipid oxidation is also reported as the primary contributor to flavor formation in pulse ingredients (Sessa & Rackis, 1977; MacLeod et al., 1988; Reineccius & Heath, 2006). As indicated in Fig. 5, the FBIC was situated along the cluster of gently processed ingredients, being strongly associated with acetaldehyde, 1-hexanol and 2-methylfuran, which are possible indicators of lipid oxidation (MacLeod et al., 1988; Reineccius & Heath, 2006). The FBIC also contained 1-R- α -pinene and 3-carene, which are products of carotenoid degradation in plants (MacLeod et al., 1988; Baldermann, Naim, & Fleischmann, 2005; Siegmund, 2015). This means that the FBIC, however very gently processed, has already undergone some level of volatile generation, in proportions that contributed to a more pronounced green odor perception.

Moving further to the concentrates that were more but rather gently processed, $\text{pH}_{\text{process}}$ 4 and 6.4 were associated to more milk, fresh and orange blossom attributes, and lied in the same quadrant as the FBIC (Fig. 5). For the $\text{pH}_{\text{process}}$ 6.4 ingredient suspensions, aldehyde and alcohol signals were predominant, with certain variations related to the extent of processing (T_{process} and t_{process}). Nevertheless, the volatile proportions were similar to that of the FBIC suspensions at $\text{pH}_{\text{utilization}}$ 4 (Fig. 3). For the $\text{pH}_{\text{process}}$ 4 series, aldehyde signals followed by those of terpenoids were predominant in most of the ingredient suspensions (Fig. 3). Terpenoids, along with certain alcohols and aldehydes, are popularly studied with regard to the aroma of fruits (El Hadi, Zhang, Wu, Zhou, & Tao, 2013). Terpenoids such as 1-R- α -pinene, 3-carene and (*Z*)- β -farnesene, which were associated with these two series, have been identified to give a fruity, orange, citrus, sweet and milky aroma (Garg et al., 2017). Along with many other terpenoids derived from carotenoid degradation, they are found mainly in citrus oils (Reineccius & Heath, 2006). Therefore, in addition to lipid oxidation reactions, carotenoid degradation could also have been favored by these

processes, rendering a perception that deviated slightly from the FBIC suspensions (Fig. 5).

Now, moving towards an even higher degree of processing, the perception was driven towards a more cooked one, where two distinct zones were defined by $\text{pH}_{\text{process}}$ 2 and 11 (Fig. 5). Processing at $\text{pH}_{\text{process}}$ 2 was associated more towards red wine, meat and ammoniac notes, whereas $\text{pH}_{\text{process}}$ 11 was related to smoky and burnt odor attributes and to even T_{process} 95 °C and ‘High’ t_{process} conditions (Fig. 5). Although raw pulses give a rather green/grassy perception, bean processing at higher temperatures leads to a decrease in grassy notes, with an evolution towards nutty, roasted and cooked odor. Similar results have been obtained in previous studies (Mishra et al., 2017; Ong & Liu, 2018). Thermal treatment has been strongly associated with the generation of burnt, cooked, smoked and baked aroma – which was also seen for the concentrates modified at higher temperatures ($T_{\text{process}} \geq 75$ °C) (Monte & Maga, 1982; Reineccius & Heath, 2006; Rababah et al., 2012). In other studies, for fava bean flours used in the fortification of corn chips, the degree of processing led to the formation of burnt notes within defined acceptable levels (Rababah et al., 2012). With regard to the cooked flavor-associated reactions that are accelerated by the T_{process} , vigorous conditions of extreme pH such as $\text{pH}_{\text{process}}$ 2 and 11 have been associated with Maillard reaction, lipid oxidation, protein and sugar degradation reactions (MacLeod et al., 1988; Reineccius & Heath, 2006). Ingredient modification at $\text{pH}_{\text{process}}$ 2 was also associated with very high volatile complexity (Fig. 4 and Fig. 5). Earlier reports have shown that acidic processes in systems rich in proteins lead to protein hydrolysis, where a lower number of hydrophobic interactions decrease the opportunity of flavor binding and thus enable higher flavor release (Ng, Pascaud, & Pascaud, 1987; Reineccius & Heath, 2006; Žilić, Akiljoğlu, Serpen, Barać, & Gökmen, 2012). Examples of this were seen in the higher generation of volatile compounds for $\text{pH}_{\text{process}}$ 2 ingredients (Fig. 4 and Fig. 5). Furfural is formed by either Maillard reaction (Amadori or Heyn’s products) or from sugar degradation, and is often associated with fragrant, bread, woody, sweet, baked and almond attributes (Garg et al., 2017). Vigorous processes resulted in a higher furfural formation, suggesting the advent of those reactions and generation of associated odor attributes (Fig. 5). Maillard reaction products also include aldehydes, ketones, sulfur compounds and pyrazines that have been found to impart a “burnt” flavor in soy, along with certain Strecker aldehydes and furanoids (MacLeod et al., 1988). Furanoids (2-alkylfurans) such as 2-butylfuran and 2-pentylfuran are known to be formed from PUFA oxidation products in different pulses (MacLeod et al., 1988; Reineccius & Heath, 2006; Akkad, Kharraz, Han, House, & Curtis, 2019). The generation of these compounds in this study has been favored by the vigorous processes (Fig. 5). Formation of the products from lipid oxidation and Maillard reaction are impacted by water activity, which was the case of the reaction systems (containing 80% moisture) used in this study (MacLeod et al., 1988; Reineccius & Heath, 2006). Studies on the role of water activity in the extent of such reactions are encouraged. On the other hand, in protein-rich matrices alkaline processes enable more binding of proteins with compounds such as butanal, hexanal, 2,3-butanedione and a higher release of ketones including 2-heptanone (Overbosch, Afterof, & Haring, 1991). This matrix effect could have played a role for the ingredients modified by $\text{pH}_{\text{process}}$ 11, where relatively higher signals of ketones and aldehydes were detected (Fig. 3). These ketones include products from lipid oxidation such as 2-butanone, 2-pentanone and 2-heptanone (from linoleic and α -linolenic), 3-octen-2-one (from linoleic acid) and 3,5-octadien-2-one (from α -linolenic acid). 2-butanone and 2-pentanone are also produced from thermal decomposition of amino acids (e.g. alanine, valine, isoleucine, cysteine) and sugars (e.g. glucose, sucrose) (MacLeod et al., 1988; Akkad et al., 2019). Furthermore, acidic and alkaline processing

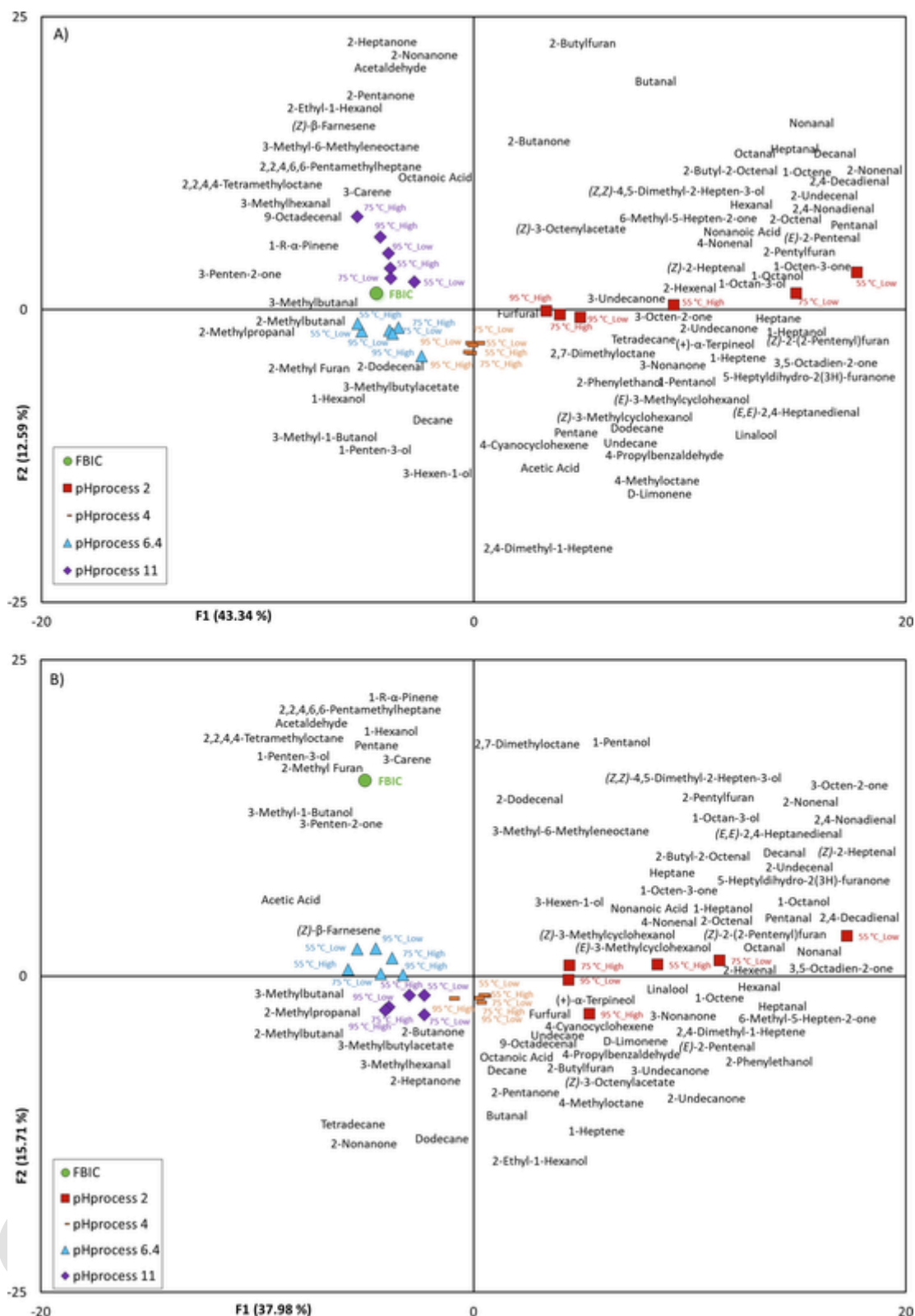


Fig. 4. PCA Projections of Volatiles Detected in Fava Concentrate Suspensions. Principal Component Analysis (PCA) projections of the volatile compounds extracted from the headspace of different ingredients suspensions by automated solid-phase micro-extraction (HS-SPME) and analyzed by gas chromatography coupled with mass spectroscopy (GC-MS). This analysis was performed on the volatile peak areas that were normalized by the d7-heptanol peak areas of their respective chromatograms and the ingredient dry weights measured for each chromatographic analysis. The volatiles were released from suspensions of different ingredients including fava bean initial concentrate (FBIC; green, circular point) and the same modified by pH: 2, 4, 6.4 and 11 (pH_{process}), temperature: 55, 75 and 95 °C (T_{process}) and treatment duration: 30 and 360 min (t_{process}), and then utilized at application pH: A) pH_{utilization} 4 and B) pH_{utilization} 7. The points on the plot represent suspensions from ingredients processed at pH_{process} 2 (red, square points), pH_{process} 4 (orange, rectangular points), pH_{process} 6.4 (blue, triangle points), and pH_{process} 11 (purple, diamond points).

have decreased due to ingredient modification conditions. Thermal treatments above 70 °C for 2 min have been known to inactivate BBL, suggesting a high sensitivity of this enzyme to process conditions (Al-Obaidy & Siddiqi, 1981). Therefore, it would be interesting to complete this study by looking into possibilities of BBL activity in the FBIC and evolution of this activity with the degree of processing, as well as conditions of utilization. Furthermore, in protein-rich systems such as the ones studied here, protein unfolding and resultant precipitation at pH close to their isoelectric points may impact the release of certain molecules compared to another condition favoring the native proteins (Reineccius & Heath, 2006). For fava bean proteins, the isoelectric pH is between 4 and 5, and thus the accentuated differences between $pH_{\text{utilization}}$ 4 and 7 can be due to protein precipitation (Sharan, Zanghelini, et al., 2021; Sharan, Zotzel, et al., 2021).

Finally, process conditions affected the complexity of the volatiles through different reactions. The effect of pH was predominant and governed the changes in the matrix, along with the types of flavor imparting reactions that could occur. This was followed by the effects of T_{process} and t_{process} , which may have determined the degree to which these reactions can take place – thus driving odor in many possible directions (Fig. 5). Odor perception and volatile chemistry are both very complex in nature, interdependent and also dependent on process conditions. Fig. 5 explained 43% of the interplay between odor perception, volatile chemistry and process conditions, suggesting that only a part of the whole phenomena has been explained, arising from heterogeneity in the different evaluation methods. There is therefore a need to have higher variety and degree of volatile and sensory studies that could identify and quantify odor-active molecules and establish their role in each of the essential odor attributes (Jourjon, Symoneaux, Thibault, & Reveillere, 2005; Cosson, Souchon, Richard, Descamps, & Saint-Eve, 2020).

5. Conclusions

Fava bean is a multi-component system containing several precursors for flavor formation. Process conditions drove changes in odor perception in protein-rich fava bean concentrates. Initially, the unmodified concentrate had stronger green notes, but this evolved into more cooked notes with the extent and type of processing – mainly governed by the pH during concentrate modification. This effect was confirmed with headspace volatile analysis of ingredient suspensions. Aldehyde signals were predominantly detected in the headspace of ingredient suspensions. Furanoids, terpenoids, alcohols and ketones signals had the next higher contribution for modifications at pH2, 4, 6.4 and 11 respectively. From gentler to vigorous process conditions, perception can be modified from more green to more cooked flavors, whereas different conditions of application (e.g. pH) can modulate between “sweet” and rancid perceptions. The utilization pH was predominant in driving odor perception, especially for the unmodified concentrate, which also showed drastic changes in volatile groups at different pH. Ingredient modification reduced this pronounced impact by pH to a certain extent. Lipid oxidation was considered an important subject matter for fava bean associated flavor, along with other reactions involving protein, sugar or carotenoid degradation. Further studies looking into the nature of these reactions would be valuable. The role of enzymes in imparting flavor during utilization conditions, especially for unmodified concentrates, needs to be further investigated. Just as the odor perception is driven by process conditions, potential modifications in taste and color of such promising ingredients need to be assessed along with process conditions in a similar approach – in which perception is explained along with food chemistry.

Uncited references

McHugh (2011).

CRediT authorship contribution statement

Siddharth Sharan: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Gabriela Zanghelini:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Aurélia Pernin:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Nicolas Descharles:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Jens Zotzel:** Resources, Writing – review & editing, Supervision, Project administration. **Daniel Bonerz:** Methodology, Validation, Formal analysis, Investigation, Data curation, Resources, Writing – review & editing, Funding acquisition. **Julian Aschoff:** Resources, Writing – review & editing, Funding acquisition. **Marie-Noëlle Maillard:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Anne Saint-Eve:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare potential conflict of interest. Jens Zotzel, Julian Aschoff and Daniel Bonerz work at Döhler GmbH. Döhler GmbH is a global producer of natural ingredients, ingredient systems and integrated solutions, including plant-based products containing fava bean. Döhler GmbH is a member of the European Union’s Horizon 2020 research and innovation program, grant agreement no. 765415 (acronym FOODENGINE) and hosted two PhD fellows, including Siddharth Sharan.

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Data Availability Statement

The raw data has been shared in Zenodo data repository supported by the European Commission (Framework Programme 7/ Horizon 2020).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2022.111582>.

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