

Characterizing the Impact of Soaking and Germination on Yellow-Eyed Bean Flour

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RESEARCH

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ABSTRACT

Dried beans are an excellent source of protein and soluble fiber but the presence of off-flavour compounds limits their widespread application as alternate flour sources in food products. Soaking and germinating beans may be effective in modifying the aroma profile of beans but their effectiveness must be demonstrated. The effectiveness of soaking and germination to modify the relative abundance of aroma active compounds (AACs) were compared in yellow-eyed (YE) bean flours using a gas chromatograph mass spectrometry (GC-MS) approach. Beans were or were not germinated for 72 h, then the beans were freeze-dried, and ground to flour using a kitchen mill. The effect of sanitization, scarification and a combination of these two methods on percent germination was also evaluated. The impact of these treatments on the chemical compositions of the flour samples were also

evaluated by measuring the protein, fat, and starch contents, and observing the electrophoretic pattern of extracted proteins. Soaking and germination resulted in different AACs as compared to the ungerminated samples. For example, hexanal was the most abundant volatile in germinated samples (25.2%) whereas in flour from soaked and ungerminated beans, the relative abundance of hexanal was 4.6%. The starch content was significantly higher ($P < 0.05$) in flour from soaked beans that were ungerminated compared to flour samples from germinated beans. However, germination did not impact the total protein and fat contents across all samples tested. These findings are important for improving processes that impact the aroma profile and the chemical composition of YE bean flours.

Key Words: Yellow-eyed bean; Aroma-active compounds; Dried beans; Gas chromatograph mass spectrometry.

INTRODUCTION

Anticipated population growth and the impacts of climate change challenge us to think differently about how we utilize our current food supply [1, 2]. Utilizing ingredients that are rich in nutrients and low in fertilizer requirements has been suggested as a course of action to meet the demand for more sustainable food products. Legumes such as beans are especially good candidates because they are high in protein (20-30%) and soluble fiber (5%), and have the unique ability to fix nitrogen [3]. This natural ability decreases their requirement for fertilizers, which in turn contributes to their low carbon footprint [3]. There is also increasing evidence to support the proposition



that diets rich in beans promote health by lowering the risk of cardiovascular disease, various cancers, and diabetes [4].

Although dried beans (*Phaseolus vulgaris*) are one of the most common types of legumes grown in Canada their consumption in North America is still low relative to countries such as India and Lebanon where pulses are a major part of the diet [5]. In order to increase the nutritional content of their gluten-free (GF) processed foods some food manufacturers have started to incorporate bean flours as ingredients to replace low-protein flours (for e.g., corn and rice) that are commonly used in GF products [6]. However, the use of bean flour in GF products has had limited success mainly because of the presence of undesirable impacts on the texture and the presence of volatile aroma-active compounds (AACs) that are considered as off-flavours in these products [7].

Volatile AACs are low-molecular-weight (< 400 g/mol), organic compounds including alkanes, aldehydes, alcohols and ketones, and they play an important role in determining the flavour attributes of many foods [8,9]. AACs and off-flavours in leguminous plants may develop by low lipoxygenase activity (during processing or storage) or maybe inherent in the plant [10]. Inherent off-flavours and AACs cannot be removed but can be modified or masked using strategies such as soaking and germination [11].

By using yellow-eye (YE) beans, we obtained support for the hypothesis that a combined approach of soaking and masking with chocolate was a successful strategy to reduce off-flavors in cake-style brownies [12]. Based on this recent work, it was further hypothesized that germination would impact the chemical profile of AACs present in flour generated from these treated beans. Germination is an inexpensive processing technique that has been used to modify the flavour profile of other plant sources including lupin [13]. However, the effectiveness of this technique to modify the composition of AACs in Nova Scotian (NS) yellow-eyed (YE) beans remains to be evaluated. Here we applied a headspace solid-phase microextraction (HS-SPME) gas chromatography-mass spectrometry (GC-MS) approach to elucidate the profile of AACs in raw (UnYE-UG) and pre-treated (SYE-G) bean flours.

The UnYE-UG flour samples were obtained from YE beans that did not undergo any pre-treatment prior to milling. Conversely, SYE-G flours were generated from YE beans that had been soaked in water then germinated and dried before milling to flour. Beans were also scarified (ScYE-G) and sanitized (SnYE-G) to evaluate the impact of these pre-treatments on the rate of germination and the chemical composition of the resulting flour samples. Our own observations have shown that YE beans have relatively soft seed coats thus making them good models in which to characterize these various pre-treatments.

The increasing demand for high-quality plant-based ingredients emphasizes the importance of this study since understanding the different processing factors that impact the flavour profile of NS bean flour has the potential to expand their utilization in new and traditional food products; which in turn will increase the value of Canadian crops.

MATERIALS AND METHODS

Materials

Nova Scotian YE dried beans (*Phaseolus* spp. L.) were purchased from a grocery store in Antigonish, Nova Scotia. Total Starch Assay Kits were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland), and all other chemicals used were obtained from Sigma-Aldrich Canada (Oakville, Canada).

Methods

Pre-treatments of Beans

To evaluate the impact of soaking and germination on aroma profile and chemical composition the following bean samples were prepared. First, untreated, raw YE beans that did not undergo any pre-treatment, were milled to flour, and are described as (UnYE-UG). Second, YE beans that were soaked, freeze-dried and milled to flour, are described as (SYE-UG). The third category of beans were soaked, germinated and then freeze-dried before milling to flour, and are described as (SYE-G). Beans that were soaked were placed in distilled water (dH₂O) at an 1:5 ratio of beans to dH₂O for 12 h prior to germination.



Bean samples were also prepared to investigate the impact of sanitization, scarification, and a combination of these two pre-treatments on the rate of germination and the chemical composition of the resulting bean flours. Sanitized beans were soaked in 0.07% sodium hypochlorite for 30 min, then rinsed with distilled water until a pH of 7 was achieved, and are described as (SnYE-G). Beans to be scarified had 2 mm nicks cut into their seed coats, and are described as (ScYE-G), whereas seeds that were both sanitized and scarified are described as (Sn/ScYE-G). All pre-treated beans were soaked as described above prior to germination. Beans with no pre-treatment (untreated) were also germinated and used as a control (UnYE-G).

Bean Germination and Flour Preparation

YE treated and untreated beans were germinated using modified versions of the methods described by Ma, Boye and Hua (2018) and Xu, Jin, Lan, and Rao (2019) [10,14]. Twenty-five beans were placed into a petri dish lined and covered with moist filter paper. Beans were germinated in quadruplicate in a Memmert Humidity Chamber (HCP50) at 25 °C and 95% RH for 24 h to 72 h. On each day, germinated beans, defined as beans with a radicle of longer than 2 mm, were removed and their radicle length was measured, and the remaining beans were rinsed with dH₂O to maintain moisture. Percent germination will be calculated using the following equation:

$$\% \text{ germination} = \frac{\# \text{ of germinated seeds}}{\text{total seeds}} \times 100$$

At the end of the germination period, all the germinated beans were freeze-dried and ground into flour using a Blendtec 51-601-BHM Kitchen Mill on the finest setting. Flour samples were sifted through a stainless-steel mesh sieve (0.212 mm) and stored in airtight containers at 4°C prior to analysis.

Determination of Proximate Composition

Crude protein and lipid analysis of all the flour samples were determined at the Department of Agriculture

and Food Operations Laboratory, (Nova Scotia Department of Agriculture). Protein content was determined by a combustion analysis method (Laboratory Services Analytical Laboratory, LSAL, Method 410) using a LECO CN828 macro combustion instrument (St. Joseph, United States). Conversely, crude fat was determined by a solvent extraction method. The total starch content of each flour sample was analyzed using a Megazyme assay kit K-TSTA-100 A (Wicklow, Ireland). The amount of glucose produced by the hydrolysis of starch was estimated by measuring the absorbance at 510 nm using an Ultrospec® 2000 spectrophotometer as previously described by English, Viana, and McSweeney (2019) [12].

Protein extraction

Protein extractions from all flour samples were carried out using a modified version of the method described by Wallace et al. (1990) [15]. Protein was extracted overnight from flour samples (100 mg) dispersed in 2 mL borate buffer (0.1M, pH 10) at 37 °C. The samples were centrifuged at 1500 x g for 15 min and the supernatant was collected and filtered through 0.22 µm, MCE, sterile syringe filters (Millipore Sigma). The protein concentration of the obtained extracts was determined using the Bradford assay, and the excess samples were aliquoted into smaller volumes and stored at 4°C prior to analysis [16].

SDS-PAGE

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the methods described by English et al. (2109) and Laemmli (1970) [12, 17]. Protein extracts (15 µg) were mixed with 5 µL of protein sample buffer and heated for 10 min at 100 °C. Fifteen microlitres of each denatured protein extract was loaded onto a 12% Mini-PROTEAN® TGX™ gel (BioRad, Mississauga, ON). Gels were loaded into a Bio-Rad Mini-PROTEAN® Tetra Cell (Mississauga, ON) and the system was run for 40 min at 170 V and 30 mA. The gels were stained for 2 h with 0.1% Coomassie Brilliant Blue (R-250) solution and de-stained, and then gel images were captured using a BioRad Chemic Doc™ MP imaging system. A pre-stained



protein marker (New England BioLabs, P77066) was used to estimate the molecular weights of proteins in the samples.

Headspace-Solid-Phase Microextraction (HS-SPME) Gas Chromatography x Gas Chromatography-Time of Flight Mass Spectrometry (HS-SPME-GCxGC-TOFMS)

Bean flour samples described as UnYE-UG, SYE-UG, and SYE-G were each combined with high purity water in a 1:1 ratio mass-based mixture in a small beaker at room temperature. The samples were mixed for 1 min and 1 g was weighed into a 20 mL headspace vial. Vials were capped and incubated for 24 h at 37 °C (Isotemp incubator, Thermo-Fisher Scientific). The samples were replicated twice. Analyses were performed using an Agilent 6890N GC (Agilent Technologies) coupled to a Leco Pegasus 4D TOF-MS (Leco Corporation, St. Joseph, MI, USA). Samples were incubated for 3 min at 30°C with a Gerstel MPS autosampler (Gerstel, Linthicum, MD, USA). A microextraction fiber (1cm) with a divinylbenzene/carboxen/polydimethylsiloxane 50/30 µm coating (Supelco, Sigma-Aldrich, Canada), was used to extract volatiles for 30 min, and the fiber was transferred to the injector port for 3 min desorption at 250 °C in splitless mode.

Helium was used as the carrier gas at a flow rate of 1.4 mL/min. Volatiles were separated on a Stabilwax 30 m × 0.25 mm, 0.25 µm film (Restek, Bellefonte, PA, USA) column in a first dimension (1D) and a Rxi-5Sil MS 2 m × 0.25 mm, 0.25 µm film thickness (Restek, Bellefonte, PA, USA) column in the second dimension (2D). These columns were connected with a dual-stage quad-jet thermal modulator. Oven temperature was held at 50°C for 0.2 min and ramped at a rate of 10.3 °C/min to 220 °C. The secondary oven temperature was held at 33 °C above the temperature of the primary oven throughout the chromatographic run. The modulator was offset by +15 °C in relation to the secondary oven and the modulation time was 1.30 s, with a hot pulse time of 0.39 s. Transfer line and the ion source temperatures were set at 250 °C and electron ionization at 70 eV with an optimized offset of 200 Volts. After an acquisition delay of 30 s, spectra were collected with a mass

range of 35–400 m/z with an acquisition rate of 200 spectra/s.

GC × GC-TOFMS Data Alignment and Processing

Statistical Compare in ChromaTOF software version 4.72 was employed to align and process chromatograms. The baseline offset was set at 0.5 and signal to noise ratio (S/N) was at 20. The 1D peak width was set to 7.8 s while the 2D peak width was set to 1.3 s and traditional integration was used. The required match to combine peaks was 700, applying a mass spectral library (NIST 2017). Mass threshold was set at 10 and the minimum similarity to assign a compound name was set at 600. The retention time match for the maximum number of modulation periods was 1.

Statistical Analysis

The relative abundance of each volatile compound was expressed as a percentage of the total peak area of all volatile compounds after background subtraction, which allowed a semi-quantitative comparison to be made [18]. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was performed on the data using GraphPad Prism version 8.30 for MacOS, GraphPad Software, La Jolla California USA, www.graphpad.com.

RESULTS AND DISCUSSION

The effect of different processes on the chemical composition of YE bean flour

In the experiments investigating the percent germination, only the beans described as (SYE-G) had radicles 2mm or longer after 24 h (Figure 1), and the cumulative percent germination for these beans would be given as 56.4%, 85.4% and 89.4% after 24 h, 48 h and 72 h, respectively. Conversely, the cumulative percent germination exhibited after 72 h was lower in beans that were scarified (ScYE-G), and those that were given the combined pre-treatment of sanitization and scarification (Sn/ScYE-G), 36% and 39%, respectively.



Moisture is a key requirement for germination, and is required to hydrate cell contents, activate enzymes, and breakdown and translocate reserve storage materials [19]. Thus, beans that are fully hydrated are more likely to initiate the germination process under the appropriate conditions. This was clearly demonstrated with the SYE-G beans. On the other hand, untreated beans and those that were sanitized prior to germination showed the highest germination percentages after 72 h, 23% and 29% respectively (Figure 1). In these two instances limited available water and the presence of chlorine may have inhibited the rate of germination and thus provide an explanation for the delay in germination. In the present experiment scarification did not appear to increase percent germination in the tested beans, however, in other studies this technique was successful in increasing the percent germination of *Lathyrus* seeds [20].

However, Miano and Augusto (2018) noted that the hydration of leguminous seeds is not a simple process, and can be affected by other factors such as seed structure and composition which can impact overall hydration kinetics behaviour [21]. In theory, hydration kinetics may be characterized by rapid hydration at the beginning of the process with a gradual decrease in hydration rate until an equilibrium moisture content is achieved. An alternative hypothesis proposes that hydration kinetics may be slow at the start of the process, and then accelerates up to a certain inflection point, and then the rate decreases until an equilibrium moisture content is achieved [21]. This raises the possibility that beans from the various groups studied used different mechanisms of hydration kinetics which would also play a role in determining the rate of germination.

To assess whether the various treatments impacted the chemical composition of YE beans we measured the protein, fat, and starch contents of the corresponding flour samples. The impact of the treatments on the total protein content of the flour samples appeared negligible and the average values registered were 24.8 ± 0.5 (% dry basis). A similar trend was observed for the fat content with an average value of 1.8 ± 0.3 (% dry basis)

recorded for the flour samples tested. During germination key biochemical reactions including the weakening of seed covers and the production of specific enzymes are initiated to aid in the development of the growing seed [19]. The increase in proteolytic enzymes is believed to assist in the mobilization of reserves from storage proteins to the growing seed. However, other researchers have noted that while germination results in an increase in protein hydrolysis there are negligible changes observed in the total protein content since the protein content depends on a balance between protein degradation and protein biosynthesis during germination [21]. This could provide a possible explanation for the negligible changes observed in the total protein content in the present study. In addition, the electrophoretic profiles did not show a significant number of small peptides, which further supports the observations noted with the protein content (Figure 2). There was however, a protein band (~55 kDa) present in the soaked flour sample that was not observed in the other flour samples (Figure 2). In a previous study we have shown that this protein band is also present in untreated, ungerminated YE beans (UnYE-UG) [12].

On the other hand, the starch content measured from the SYE-UG and UnYE-G samples varied significantly ($P < 0.05$) as compared to the other samples (SYE-G, SnYE-G, ScYE-G and Sn/ScYE-G) for which an average starch content of 27 ± 2.1 (% dry basis) was recorded (Table 1). Germination also facilitates the enzymatic breakdown of starch into simple sugars by activating enzymes such as α -amylase [19], which explains why the ungerminated samples in the present study had the highest amount of total starch on a (%) dry basis (Table 1).

The role of pulses including beans in health promotion is gaining interest partly because of the increased consumption of plant-based diets Chardigny and Walrand (2016) [22]. The consumption of pulses has also been associated with preventative effects in some chronic conditions including diabetes and hypercholesterolemia Abeysekara, Chilibeck, Vatanparast, Zello (2012) [23]. In addition, proteins from plant sources provide a cheaper protein supply, and have lower agricultural inputs (Sabaté,



Sranacharoenpong, Harwatt, Wien, Soret, 2015; English, 2019) [24, 26]. Other researchers have also proposed the blending of plant and animal protein sources as a step to improve the sustainability of our current food supply Alves and Tavares (2019) [26].

In the present study, the stability of the protein content in flour samples even after various pre-treatments of the beans (Table 1), helps to provide evidence to validate the potential application of these flours to improve the nutritional properties of gluten-free products. Although the use of bean flours may present many food product development and health management opportunities, the presence of off-flavour compounds in these ingredients still needs to be addressed.

The effect of soaking and germination on the profile of AACs

The potential of various pre-treatments of YE beans to modify the aroma-profile of subsequent flour samples was evaluated using GC-MS. The twelve most abundant AACs from the more than 100 compounds detected in flour samples generated from beans that were untreated (UnYE-UG), soaked (SYE-UG), and those that were soaked and then germinated, (SYE-G) are shown in Table 2. Ethanol was common to all three samples, whereas hexanal was found in both the SYE-UG and the SYE-G bean flour samples, but at a higher relative abundance in the latter, 4.6% as compared to 25.2%. As noted earlier, the fat content of the flour samples did not appear to be affected by germination, however, hexanal can be formed through the oxidation of linoleic and oleic acids, which are components of the fats found in bean flour [27, 28]. Toluene was absent from the UnYE-UG samples, but present in both the SYE-UG and the SYE-G samples, and at a relative abundance of 6.2% and 4.6%, respectively. There were also three unknown compounds present in the germinated samples with relative abundances of 7.5%, 5.6%, and 3.3%, and one in the soaked samples with a relative abundance of 6.4%.

The different aroma profiles obtained from treated flour samples in the present study add to the emerging

evidence that soaking and germination can be used to modify the aroma profile of pulses [14]. However, sensory evaluation studies are needed to identify the volatiles that are contributing to the undesirable aroma. Although most studies using pulses have investigated the effectiveness of these treatments on the functional properties of pulse flours, the present study provides new knowledge about the combined effect of these two treatments on the aroma profile of YE bean flours. It has been shown that the headspace SPME GC-MS method was a suitable approach to identify volatile AACs in the YE flour samples tested.

The low abundance of some of the compounds identified may be attributed to the fact that no optimization experiments were performed to optimize analyte enrichment. Indeed, several factors including the type of fiber, the fiber extraction time, and the temperature used for the extraction of volatiles can affect volatile compound extraction [14]. In the present study only one type of SPME fiber was used, but other researchers have shown that fibers behave differently in terms of their ability to absorb target volatiles [14]. Thus, performing these optimization experiments is one way to improve future studies that use a GC-MS approach to examine AACs in YE beans.

Pulses can be processed in different ways and can undergo various pre-treatments to improve their aroma profile. However, these processes have the potential to either improve or degrade the nutrient quality and the functional properties of ingredients generated from processed pulses. To better understand these changes, future research should continue to evaluate the functional properties of proteins generated from these raw ingredients. This research will help to identify appropriate conditions for processing techniques that will help contribute to a high-quality ingredient supply.

CONCLUSION

A headspace SPME GC-MS approach was used to identify the relative abundance of AACs present in flour samples generated from beans that were untreated (UnYE-UG), soaked (SYE-UG), and soaked and then germinated, (SYE-G). Hexanal was the most volatile compound identified



in the SYE-G samples, whereas ethanol was present in all three samples. It has been shown that soaking and germination can result in different profiles for AACs present in these flour samples. However, sensory evaluation studies would be required to determine whether these differences are desirable or undesirable. It was also shown that germination did not affect the total protein and fat contents in the samples studied. In addition, soaking appeared to be the most effective method to increase the percent germination in the samples studied. Future experiments will optimize the conditions for analyte enrichment and seek to match flavour descriptions with the volatile compounds identified.

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FIGURES

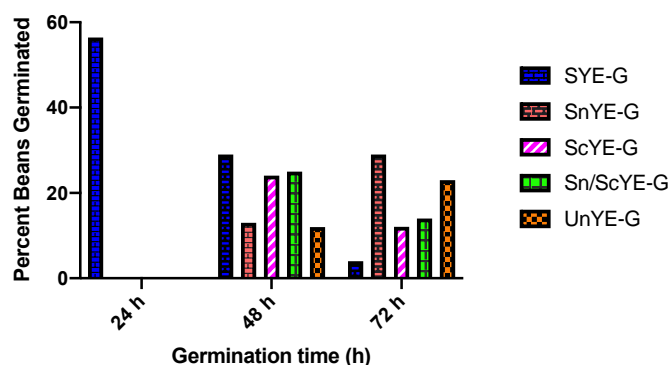


Figure-1. Percent YE beans germination over 72 h. After 24 h approximately 56.4% germinated beans were observed in the SYE-G samples. Germinated beans were observed for all the samples at the 48 h and 72 h time points. Cumulative percent germination was highest for the SYE-G beans (~89%), whereas the other samples had an average cumulative percent germination of $38\% \pm 3.2$.

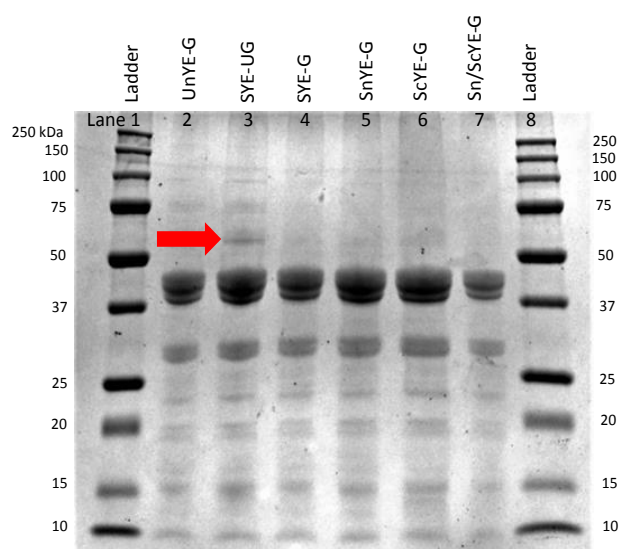


Figure-2. SDS-PAGE showing molecular weight distributions of proteins extracted from untreated (UnYE-UG) and pre-treated (soaked, sanitized, or scarified, and scarified and sanitized) YE beans prior to germination are shown in lanes

2, 4, 5, 6 and 7, respectively. Proteins from YE beans that were soaked but not germinated (SYE-UG) are shown in lane 3, and this sample has a protein band (~55 kDa) that is not observed in the other flour samples. All protein samples were separated using a 12% polyacrylamide resolving gel and visualized using a Coomassie Brilliant Blue stain (0.1%). A New England BioLabs (P77066) pre-stained protein marker ranging from 10-250 kDa (lanes 1 and 8) was used for these studies.

TABLES

Table-1. Total protein, fat, and starch content of flour samples expressed as percent dry basis. The protein and fat contents appeared to be similar with no significant differences between samples. However, the total starch content of flour samples generated from the UnYE-G and SYE-G beans varied significantly ($P < 0.05$) compared to the other pre-treated samples.

Flour samples	Total Protein (% dry basis)	Total Fat (% dry basis)	Total Starch (% dry basis)
UnYE-G	24.4	1.6	35.3*
SYE-UG	24.2	2.0	42.9*
SYE-G	24.9	1.7	27.9
SnYE-G	25.7	1.8	28.2
ScYE-G	24.7	1.5	23.7
Sn/ScYE-G	24.7	2.2	27.6
Average values	24.8 ± 0.5	1.8 ± 0.3	27 ± 2.1

*For total starch, these values are not included in the average estimates.

Table-2. The twelve most relatively abundant headspace volatile compounds in untreated YE beans that were milled to flour (UnYE-UG), YE beans that were soaked, and milled into flour (SYE-UG), and in YE beans that were soaked, then germinated before being milled to flour (SYE-G). Ethanol was common to all three samples, whereas hexanal was only found in the SYE-UG and the SYE-G bean flour samples, but at a higher relative abundance in the latter, 4.6% as compared to 25.2%, respectively. There were also three unknown compounds in the germinated samples.

<u>UnYE-UG</u>		<u>SYE-UG</u>		<u>SYE-G</u>	
Names of Compounds	Relative Abundance (%)	Names of Compounds	Relative Abundance (%)	Names of Compounds	Relative Abundance (%)
Ethanol	36.3	Ethanol	18.2	Hexanal	25.2
Acetaldehyde	17.1	Ethylcyclopropane	8.5	3,5-Dimethyl-1-hexene	9.2
2,3-Butanediol	14.9	2,4-Dimethyl-1-heptane	6.9	Ethanol	7.9
Acetic acid	14.6	3,5-Dimethyloctane	6.7	3-Methylbutanal	7.8
2-Oxo-propanoic acid	5.1	Undecane	6.5	Unknown	7.5
R-(-)-Cyclohexylethylamine	3	Unknown alkane	6.4	(Z)-2-Penten-1-ol	6.7
Diisopropyl amine	1.2	Acetone	6.3	Unknown	5.6
1-Methoxy-3-methylbutane	1.1	Toluene	6.2	Ethylbenzene	5.1
Methyl glyoxal	0.6	2,3,4-trimethylhexane	6.2	Pentanal	4.9
Cyclobutylamine	0.6	3,7-Dimethyl-1-octene	6	Toluene	4.6
2,3-Pentanedione	0.5	4-Methyloctane	4.6	Unknown	3.3
3-Methyl-1-butanol	0.5	Hexanal	4.6	Butanal	3.3

PEER REVIEW

Not commissioned. Externally peer reviewed.

