



# A possible solution to minimize scotta as a food waste: a sports beverage

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Please find enclosed the revised manuscript: **\*A possible solution to minimize scotta as a food waste: a sports beverage**" by Tirloni Erica, Vasconi Mauro, Cattaneo Patrizia, Moretti Vittorio Maria, Bellagamba Federica, Bernardi Cristian and Stella Simone.

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Review

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Thanking you in advance for your attention,

Erica Tirloni

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8 9 10	3	Running head: Scotta as a beverage			
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# 15 Abstract

A pilot study-trial was performed on ready to drink beverages produced in a small-scale dairy plant starting from Ricotta whey (scotta) with the addition of fruit puree and starter cultures. Microbiological shelf-life was evaluated at 4 and 12°C. At 4°C the product showed moderate total viable counts until the end of the trial (~5 log cfu/mL). Yeasts proved to be the specific spoilage microorganisms of the product. When applying a thermal abuse at 20°C for 6h, TVC did not increase. The addition of fruit puree changed the volatile profile of the beverages compared to raw scotta.

## 24 Keywords:

25 Dairy microbiology; Microbiology; New product development; Shelf life; Whey; Yeasts.

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#### Introduction

Dairy products, although providing all the crucial nutrients being included in a daily balanced diet, have a negative impact on environment in terms of resource disposal and greenhouse emissions. Moreover, dairy industry produces a major part of liquid wastes (Gonzales-Garcia et al. 2013). It is considered that worldwide whey production is around 180-190 million tons/year, with an increase of 2% per year (Mollea et al. 2013). 

Wastes and wastewaters deriving from dairy industry are characterized by the presence of complex contents difficult to degrade such as lactose and in minor part proteins, lipids, vitamins, salts, and pollutant remains like chemicals deriving from cleaning procedures (Palmieri et al. 2017; Ahmad et al. 2019). 

26 36 Cheese whey is a yellowish by-product fluid obtained from cheese-making and casein 28 <del>37</del> manufacture in the dairy industry; it is the residual liquid remaining after curd separates from milk following caseins' coagulation (Zadow 1994). Scotta is the end-product of Ricotta cheese, 30 <del>38</del> a dairy product obtained from cheese whey after acid-heat coagulation and precipitation of whey proteins. In Italy, according to Sansonetti et al. (2009), about 15% of whey is used for Ricotta production, resulting in more than 1 million tons of scotta per year.

In the past, cheap and easy solutions were used by dairy producers to discard cheese whey and scotta, such as vaporization of the product on fields or elimination in rivers, lakes or the ocean. This approach is nowadays negatively felt worldwide, due to the polluting power of cheese whey; moreover, strict environmental legislations have been introduced to avoid improper dumping and to find out new opportunities to reuse these effluents (Smithers, 2008). Although having many implications as pollutants, cheese whey and scotta maintain a biological and nutritional value that are exploited only in small part. Nowadays, a minor fraction of whey is recycled and transformed in valuable products (e.g. animal feed, pharmaceutical industry, or industries which produce infants' dried powder) (Guimarães et al. 2018; Monteiro et al. 2018). Recent surveys have showed the potentiality of transformation of cheese whey into yeast bioprotein, bioethanol production, starter cultures, functional and nutritional proteins and 

bioactive peptides (Yadav *et al.* 2015; Nam *et al.* 2016; Stankey *et al.* 2017; Kaminarides *et al.* 2018).

55 Considering scotta, few studies have focused on the possible use of this substrate for 56 the production of fermented drinks (Maragkoudakis *et al.* 2006), for obtaining lactose by 57 crystallization (Pisponen *et al* 2013), or obtaining lactic acid by LAB (Pescuma *et al.* 2008; 58 Secchi *et al.* 2012) and bio-ethanol by yeasts fermentation (Sansonetti *et al.* 2009; Zoppellari 59 and Bardi 2013).

Another possibility is to explore its use for sports beverages, that are turning more often to whey proteins for their special functionality and high nutritional properties; some examples are currently on the market, although only Maragkoudakis *et al.* (2006) approached the evaluation of a potential new drink in Italy.

In the present study, a pilot production of a ready to drink beverage produced starting from Ricotta cheese whey (scotta) was considered. Shelf-life at different temperatures was determined and the potential thermal abuse was also investigated.

Materials and methods

### Beverages preparation

In the present study a microbiological shelf-life of scotta beverages obtained from the remained scotta after Ricotta production, was conducted. Two starter cultures used also for yogurt production (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) were added to scotta; these cultures were mixed into it, maintained at 42°C until the pH reached almost 4. Afterwards, the beverages were cooled at 4°C to stop the fermentation process. Then, two different commercial pasteurized purees were added (550 g for 4 L of scotta), which consisted in sugar, tropical (mango flavoured) or citrus fruits (35%), water, gelling agent: pectin E440, flavouring, preservative: E202 - potassium sorbate (0.13%), acidity regulator: sodium citrate - E331. The two types of beverage were packaged into 125 mL bottles and submitted to the subsequent tests. Each of the two series (citrus or tropical beverages) was stored at two different static temperatures (4 and 12°C).

At settled sampling times (0, 7, 14, 21, and 28 days from the production) the samples stored at 4°C or at 12°C were analysed in triplicate. 

#### Microbiological shelf-life

For microbial counting, 10 mL of each sample were homogenized in 90 mL of a diluent solution (0.85% NaCl and 0.1% tryptone), and serial 10-fold dilutions were prepared. Total 15 85 mesophilic count (TMC) excluding Lactic Acid Bacteria was determined using gelatine peptone bios agar (AG) (Biogenetics, Ponte San Nicolò, Italy), subsequently incubated at 30°C for 48 h. L. delbrueckii subsp. bulgaricus was enumerated on de Man-Rogosa-Sharpe agar (Oxoid, Basingstoke, UK), acidified at pH 5.2 according to ISO/FDIS 7889 IDF 117 standard (ISO, 2002). S. thermophilus was counted onto M17 agar (Oxoid) supplemented with lactose (5 g/L) and incubated under aerobic conditions at 45°C for 24 h (IDF, 1981); this medium, although not strictly selective, is very often applied for the enumeration of lactic streptococci in milk and dairy products. Enterobacteriaceae were enumerated by the ISO 21528-2:2017 method. Escherichia coli were enumerated according to the ISO 16649-2:2001 method. Coagulase-positive Staphylococci were determined by the ISO 6888-1:2018 method. Yeasts and moulds were enumerated according to ISO 21527-1:2008 method. Bacillus cereus was enumerated onto PEMBA agar (Biogenetics) and incubated at 30°C for 48 h (ISO 7932:2004). Salmonella spp. detection was performed by ISO 6579:2017/Cor 1:2017 methods. Finally, detection of Listeria monocytogenes was performed according to the AFNOR method (AFNOR BRD 07 / 4-09/98). 

46 100 At the same sampling times, pH was measured by a pH meter (Amel instruments, Milano, I): <sup>48</sup> 101 three independent measurements were performed on each sample and means were calculated.

51 102 Moreover, a thermal abuse by maintaining the two series of products at 20°C for 6 h 53 103 was applied, simulating the likely duration of the product by an individual during prolonged 55 104 physical activity. The same parameters reported above were considered and analyses were 57 105 performed in triplicate.

60 106 Volatile compounds analysis

The volatile compounds analysis of the experimental beverages was made using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS). Analysis was carried on raw scotta used for the preparation of the 2 sport beverages, one day after the addition of the starter culture, as described above, and after the addition of citrus and tropical flavour. Then samples of the two beverages were analysed at the end of the <sup>13</sup> 112 trial, both those kept at 4 and those at 12°C. Quickly 5 ml of scotta and experimental sport beverages were transferred in a 20 mL vial, then 1 g of NaCl was added. After that, vials were closed by a PTFE / silicone cover, and shaken for 5 minutes to allow salt dissolution. Before extraction, stabilization of the headspace in the vial was obtained by equilibration for 20 mins at 40°C, using a multi-purpose sampler MPS2 XL (Gerstel GmbH, Mülheim an der Ruhr, Germany) equipped with the SPME option. Extraction and analysis of volatile compounds were made using the methods described in our precedent work on dairy products (Tirloni et al. 2018).

# 120 Statistical analysis

Microbiological and chemical-physical results obtained at the different sampling time were subjected to statistical analysis through ANOVA test. A probability of P < 0.05 and P < 0.01 was considered as a threshold value for statistically significant differences.

**Results and discussion** 

In this study, a pilot production of a ready to drink beverage prepared starting from Ricotta cheese whey (scotta) was investigated. Two series were considered, based on the flavour of puree added after production: citrus or tropical flavour. The two series of beverages were maintained at 2 static temperatures (4°C, thus ideal refrigeration and 12°C mimicking a thermal abuse) to determine their microbiological shelf-life. To improve their functional value, starter cultures used in yogurt production (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) were also added to the beverages.

Considering *S. thermophilus* counts, the products were stable at the 2 temperatures considered with loads always above 7 log cfu/mL in all samples. *S. thermophilus* population

was stable during the storage period at the two temperatures: at 4°C in citrus flavour samples, 134 the loads ranged from 8.09 log cfu/mL (d 0) to 8.33 log cfu/mL (d 28), whereas in tropical 135 flavour samples, the loads were from 8.07 log cfu/g (d 0) to 8.39 log cfu/mL (d 28). At 12°C, 136 137 S. thermophilus, in citrus flavour samples, showed loads from 7.48 log cfu/mL (d 0) to 8.25 138 log cfu/mL (d 28), whereas in tropical flavour samples, the loads were from 8.21 log cfu/g (d <sup>13</sup> 139 0) to 8.39 log cfu/mL (d 28).

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16 140 Considering L. delbrueckii subsp. bulgaricus counts, the products showed very low loads 18 141 at T0, with counts always below 4 log CFU/mL in all samples stored at 4°C. At 12°C in citrus 20 142 flavour samples, the loads ranged from 3.41 log cfu/mL (d 0) to 7.44 log cfu/mL (d 28), <sup>22</sup> 143 whereas in tropical flavour samples, the loads were from 3.39 log cfu/mL (d 0) to 7.48 log <sup>24</sup> 144 cfu/mL (d 28). We could hypothesize that for this typology of substrate (rich in lactose but <sup>26</sup> 145 substantially poor in any other nutrient), a selection of specific Lactic Acid Bacteria would be 28 146 convenient to optimize their ability of fermentation, in order to optimize their performances.

31 147 Lactic acid bacteria were generally the predominant microflora due to the inoculation of starter 32 <sup>33</sup> 148 cultures (especially due to the ability of fermentation of S. thermophilus), as already reported 34 <sup>35</sup> 149 for dairy products like yogurt or similar, where starter cultures are added (Mataragas et al. 36 <sup>37</sup> 150 2011; Tirloni et al. 2015). 38

40 151 Considering Total Viable Count (TVC) at 4°C (Figure 1), the beverages showed low 41 42 152 loads for the whole sampling period (28 d): at 4°C in citrus flavour samples, the loads ranged 43 44 153 on average from 4.16 log cfu/mL (d 0) to 4.84 log cfu/mL (d 28), whereas in tropical flavour 45 46 154 samples at 4°C, the loads were on average from 4.31 log cfu/mL (d 0) to 5.76 log cfu/mL (d 47 <sup>48</sup> 155 28). At 12°C, TVC, in citrus flavour samples, showed final loads on average of 5.74 log cfu/mL 49 <sup>50</sup> 156 (d 28), whereas in tropical flavour samples, the final loads were on average above 6 log 51 <sup>52</sup> 157 cfu/mL (d 28). 53

55 158 Yeasts were the restrictive factor for the limitation of the product's microbiological 56 57 159 shelf-life: these microorganisms, that are also the specific spoilage organisms for yogurt, 58 59 160 showed to be able to express their metabolic activity, in terms of fermentation of lactose 60 (Jakobsen and Narvhus 1996; Mataragas et al. 2011; Tirloni et al. 2015). In particular, 161

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considering yeasts (Figure 2), the products generally showed loads below the detection limit (2 162 Log cfu/mL) at T0 except for the beverage with tropical flavour at 12°C where a low countable 163 load was detected (2.15±0.21). During the storage at 4°C a slight and gradual increase was 164 165 observed reaching final loads on average equal to 4.62 and 5.48 log cfu/mL in citrus and 10 11 166 tropical flavour beverages. During the storage at 12°C, an increase was observed reaching 12 13 167 final loads, on average, equal to 5.90 and 5.47 log cfu/mL in citrus and tropical flavour 14 15 168 beverages, respectively. A limit of 5 log cfu/g has previously been reported as a threshold limit 16 17 169 above which consumers may perceive a sensorial alteration (Suriyarachchi and Fleet, 1981): in 18 19 170 citrus flavour beverages at 4°C this limit was never exceeded, while at 4°C in tropical flavour 20 21 171 beverages this limit was overcome after only 14 days of storage. At 12°C, in citrus flavour 22 23 172 beverages the load of 5 log cfu/mL was overcome after 22 days of storage and only after 14 24 25 173 days in in tropical flavour beverages. 26

<sup>28</sup> 174 E. coli, Enterobacteriaceae, coagulase-positive staphylococci, Bacillus cereus and 175 moulds were below the detection limit (2 log CFU/mL) at all sampling times. Salmonella spp. 176 and *L. monocytogenes* were always absent in 25 g of product at all sampling times.

<sup>35</sup> 177 pH values were generally constant in the beverages at all sampling times, without any <sup>37</sup> 178 clear trend with values on average, ranging between 4.09 and 3.98 (4°C-citrus), 4.09 and <sup>39</sup> 179 3.92 (4°C-tropical), 4.07 and 3.94 (12°C-citrus) and 4.07 and 4.08 (12°C-tropical).

42 180 Considering a possible thermal abuse simulating a prolonged sports activity (in this case 43 44 181 20°C for 6h), no increase was observed in TVC in both the series (citrus and tropical) (citrus: 45 46 182  $T0=3.57\pm0.07$ , T6h=3.43±0.07; tropical: T0=3.34±0.48,  $T6h=3.53\pm0.10$ ). Yeasts, 47 <sup>48</sup> 183 Escherichia coli, Enterobacteriaceae, coagulase-positive staphylococci, Bacillus cereus and 49 50 184 moulds were at all sampling times below the detection limit (2 log CFU/mL), while Salmonella 51 <sup>52</sup> 185 spp. and *L. monocytogenes* were absent in the two sampling times. 53

55 186 The volatile organic compounds found in scotta and the two experimental beverages at T0 are 56 57 187 shown in Table 1. Data are expressed as a percentage of total ion count area of all volatile 58 59 188 compounds identified. The addition of fruit puree changed almost completely the volatile profile 60 of the beverages if compared with raw scotta. Raw scotta volatile profile was constituted 189

mainly by ketones, followed by acids and alcohols. This product is the derivation of two previous processes; the first one used raw milk in cheese production, resulting in whey, which in turn, following a second processing, produces Ricotta and its by-product, scotta. The volatile compounds found in scotta are the results of these two processing phases, where thermal treatments and oxidation produced an increase of free fatty acids and the product of their <sup>13</sup> 195 degradation, such as aldehydes and ketones. This pattern was also described by Bergamaschi and Bittante (2018), who analysed the volatile compounds profile of various dairy products, from milk to scotta, considering all the intermediate products. Authors found a progressive increase of volatile organic compounds, with the higher concentration among not ripened product found in whey and scotta, where free fatty acids, aldehydes and ketones were found at the highest concentrations. This volatile pattern is not suitable for direct scotta consumption, since these substances are associated with odour descriptors of rancid (Zabaleta et al. 2016). For instance, straight chain aldehydes, like nonanal ones, are responsible for unpleasant odour when they exceed their odour thresholds (Curioni and Bosset 2002). To correct the off flavour two experimental flavouring purees were tested. 

The beverage in which a citrus puree was added presented a volatile profile dominated by terpenes, while in tropical fruit beverages a prevalence of esters and terpenes was found. In the citrus aroma beverage, as expected, limonene represented almost 93% of the total volatile compounds isolated, while in tropical fruit beverage 2-hexen-1-ol acetate was the prevalent compound. 

Figure 3 shows the variation of diacethyl, ethyl alcohol and ethyl acetate in citrus flavour beverage (A) and tropical fruit (B) sampled at the end of the trial at 4°C and 12°C and at the beginning of shelf-life test. Ethyl alcohol increased considerably in both beverages stored at thermal abuse, reaching a total ion current (TIC) 30 and 40 times higher for tropical and citrus beverages respectively, against those maintained at 4°C. In those samples, ethyl alcohol was found in a similar concentration if compared to the one measured at T0. A similar trend, even if with a slower increase, was found in ethyl acetate concentrations: this was probably due to yeasts' metabolism. Butanoic acid ethyl ester, or ethyl butyrate, a compound typically found in 

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mature fruits, deriving from the formal condensation of the hydroxy group of ethanol with the carboxy group of butyric acid. As it derives from ethanol, its trend followed its precursor's, with an increase during the preservation period. Diacetyl is an important component in the flavour of food and it is known to be the chief component of the aroma of butter. This substance plays an important role in the formation of off-flavour aroma in beer when fermentation continues in a way that is not appropriate to the correct process (Krogerous *et al.* 2015). In dairy products diacetyl is formed by lactic acid bacteria starting from citrate or lactose, if citrate is not present in growth substrate (Hugenholtz and Starrenburg, 1992). During the storage period we didn't find a significant increase of this compound in citrus flavoured beverages, while it increased in tropical fruit beverages, without differences between the two storage temperatures. Acetaldehyde (data not shown in figure 3) increased during the trial, but no differences between the two storage temperatures were found. Acetoin derives from the degradation of diacetyl. The formation of this compound showed a different trend in the two beverages: in particular, it increased in tropical fruit beverages, especially in samples subjected to thermal abuse, while in citrus beverages it was found in higher concentration at the beginning of the trial, compared to the end of the shelf-life test.

These data should be coupled in future with further sensorial analyses attesting good sensory acceptance and consumers' expectations. As already stated by Krešić *et al.* (2010) and Ahmadi *et al.* (2018), the use of whey in dairy beverages could be a promising substitute for dairy industries, as they are considered positively by consumers.

## 238 Conclusions

From a microbiological point of view, yeasts proved to be the limiting factor for shelf-life, exceeding the threshold of 5 log cfu/mL in citrus beverages (12°C of storage) and in tropical beverages (4°C and 12°C of storage) only after 14 days of storage. The addition of starter cultures seems promising, although a specific selection of natural Lactic Acid Bacteria would increase the performances especially during the substrate fermentation. The addition of fruit puree changed completely the volatile profile of the two beverages compared to raw scotta.

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![](_page_18_Figure_2.jpeg)

60

**Citrus flavour** 

4°

4°

3

Ethyl Alcohol

Ethyl acetate

ТО

Butanoic acid, ethyl ester

Diacetyl

![](_page_19_Figure_2.jpeg)

Table1: Volatile organic compounds found in scotta and in the two experimental beverages at Т0

	Raw Scotta	Citrus flavour	Tropical fruit flavour
Σ Alcohols	3.50	1.27	2.36
Ethyl alchol	$1.10 \pm 0.07$	_,	_,
1-pentanol	1.10 = 0.07 $1.17 \pm 0.04$	$0.01 \pm 0.01$	$0.42 \pm 0.01$
3-Buten-2-ol	$1.17 \pm 0.01$		0.12 = 0.01 0.47 +0.20
1-Hevanol	$1.00 \pm 0.13$ 0.23 $\pm 0.13$	$0.14 \pm 0.01$	-
	0.25 ±0.15	0.14 ±0.01	
	-	-	$0.39 \pm 0.00$
	-	-	$1.08 \pm 0.02$
	-	$1.11 \pm 0.03$	-
Σ Aldehydes	10.//	1.94	17.60
Acetaldeyde	3.30 ±1.27	$0.01 \pm 0.00$	$0.86 \pm 0.17$
Hexanal	5.29 ±1.82	-	12.78 ±0.77
Heptanal	0.47 ±0.10	-	-
Octanal	-	0.18 ±0.25	-
Benzaldehyde	-	0.12 ±0.00	-
Nonanal	1.44 ±0.03	1.55 ±0.06	-
Decanal	0.27 ±0.00	$0.01 \pm 0.01$	3.96 ±1.25
Dodecanal		$0.05 \pm 0.03$	-
Tridecanal	$\mathbf{N}_{-}$	0.02 +0.00	_
Σ Estors	1 71	-	27 77
Ethyl acotato	1.71	_	2,,
Rutanais asid athyl actor	1 71 +0 60	-	$2.05 \pm 0.15$
2 Heven 1 al asstate	1.71 ±0.60	-	$0.43 \pm 0.02$
3-Hexen-1-ol, acetate	-	-	$0.41 \pm 0.03$
Acetic acid, hexyl ester	-	-	$1.05 \pm 0.05$
2-Hexen-1-ol, acetate	-	-	$21.03 \pm 5.36$
Propanedioic acid, diethyl ester	-	$\mathbf{O}$	$1.62 \pm 0.92$
Isoamyl isobutyrate	-	-	$0.69 \pm 0.21$
Benzil acetate	-		$0.09 \pm 0.12$
Benzoic acid, 2-hydroxy-, methyl ester	-	-	$0.41 \pm 0.03$
Σ Acids	24.83	2.07	14.14
Acetic acid	1.89 ±0.34	0.02 ±0.01	2.75 ±0.39
Hexanoic acid	0.99 ±0.30	$0.01 \pm 0.01$	-
Heptanoic acid	0.92 ±0.28	-	-
Sorbic Acid	-	$1.43 \pm 0.13$	$10.98 \pm 1.80$
Benzoic acid	$12.47 \pm 3.65$	_	$0.41 \pm 0.03$
Octanoic acid	3 79 +2 28	0 51 +0 02	_
Decanoic acid	4 76 +3 24	0.51 = 0.02 0.11 $\pm 0.00$	_
S Kotonos	4.70 ±3.24	0.11 ±0.00	12 70
2 Retories	4 00 +2 27		
Acetone	4.90 ±2.27	$0.06 \pm 0.01$	$1.36 \pm 0.15$
Diacetyl	11.// ±4.61	$0.02 \pm 0.00$	$2.73 \pm 0.08$
2 Butanone 3 methyl	$2.82 \pm 0.49$	-	$0.68 \pm 0.06$
2 Pentanone	$0.27 \pm 0.06$	-	-
2,3 Pentanedione	27.65 ±3.11	-	-
Acetoin	7.40 ±1.49	-	6.60 ±0.85
2 Heptanone	0.82 ±0.23	-	$1.34 \pm 0.00$

2				
3	2-Nonanone	$0.41 \pm 0.04$	-	-
4	2-Undecanone	-	$0.01 \pm 0.00$	_
5	2-Ondecatione S Hydrocarbons	1 10	0.01 ±0.00	
6		1.10	-	-
7	Dodecane	$0.83 \pm 0.01$	-	-
8	Tetradecane	0.27 ±0.03	-	-
9 10	Σ Others	2.04	94.61	24.90
10	a-Phellandrene	-	-	-
12	a-Pinene	-	0.26 ±0.01	-
13	4-Carene	-	-	4.06 ±0.78
14	Camphene	-	-	-
15	ß-Pinene	_	$0.04 \pm 0.01$	-
16	ß-Phellandrene	_	-	041 +013
17	2 2 Dobydro 1 9 cincolo		1 17 ±0 22	0.41 ±0.15
18		-	1.17 ±0.32	-
19	β-Myrcene	-	$0.06 \pm 0.08$	-
20	a-Terpinene	-	$0.11 \pm 0.03$	-
22	Terpinolene	-	$0.04 \pm 0.01$	-
23	d-Limonene	2.04 ±0.07	92.76 ±0.01	0.97 ±0.08
24	Beta Ocimene	-	-	0.91 ±0.05
25	cis-Linaloloxide	-	-	9.91 ±0.59
26	Terpineol	<u> </u>	0.05 ±0.00	-
27	Geraniol	-	0.04 ±0.00	-
28	Citral		$0.07 \pm 0.00$	-
29	Triacetin		-	7 62 +0 89
30	Consene		$0.01 \pm 0.00$	7.02 ±0.05
32	Copaene	-	0.01 ±0.00	
33	Caryophyllene		-	1.01 ±0.16
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