Spontaneous off flavored raw milk: develop detection methods and prevention strategies

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Abstract *(Summarize the overall project as stated in the original proposal.)*

Over the past five decades, researchers have investigated factors that influence the occurrence of 'spontaneous oxidized off-flavor' in milk; however, the conclusions from this body of work are not consistent and limit our ability to monitor and control this important industrial problem. We are proposing to define, for the first time, the causative off-flavor compounds involved to more appropriately develop analytical methods for detection of this milk quality issue based on understanding the chemistry involved. Defining the chemical nature of the causative off-flavor compounds will indicate which pathways and from which precursors these negative compounds were generated to ultimately provide an improved basis to develop a simple, rapid, robust and accurate analytical detection method as well as suggest strategies for prevention.

Background Information

Many publications on spontaneous oxidized flavor in milk have relegated the problem to a variety of conditions. These include particular levels of fatty acids present in feed [1], higher levels of trace metals present in milk [2], type of feed available to cows during different seasons [3], antioxidants accessible to the cows [4], etc. However, there has been no direct consensus on a cause of the problem. Little mention has been made of the presence or levels of certain flavor compounds on the perceived off-notes present in milk that has been flagged as "spontaneously oxidized."

Objectives

The overall objectives of the study are:

1) Characterize key off-flavor compounds in select SOF identified milk samples.
2) Investigate precursors of off-flavor compounds.
3) Develop a simple, rapid, robust method to monitor the susceptibility of raw milk prone to off-flavor development.

Materials and Methods *(Describe the experimental design, analytical methods, and statistical analysis that were used to investigate each objective in your study. These should be written so another individual may use some or all the methods in another study, or judge the scientific merit of your work. When possible, use tables and figures to display the design of experiment.)*

SOF and control milk samples were obtained from a Midwestern farm that had previously been flagged with off-flavor problems similar to SOF. The milk was pasteurized for stability, and extracts were prepared on day 0 and day 14 post-pasteurization to allow for a comparison of the flavor profiles over time. 1 L each of SOF milk and control milk was extracted with 1 L of redistilled diethyl ether. Internal standards (0.06 µL of 2-methyl-3-heptanone and 0.06 µL of 2-methylvaleric acid) were added to the stock solvent for later quantification in the neutral/basic and acidic fractions, respectively. For each sample, milk was extracted in six 250 mL Teflon-lined centrifuge bottles. The samples were shaken for an hour on an orbital shaking table, after which they were centrifuged (4500 rpm and 4°C for 20 minutes). The ether layer was carefully separated from the samples, combined, and dried over anhydrous sodium sulfate. Both ether extracts underwent solvent-assisted flavor evaporation (SAFE) to separate the volatile from the nonvolatile fractions. Next, each extract was fractionated. Each was washed with 1M sodium bicarbonate (2x55 mL) followed by sodium chloride (2x55 mL). The remaining

ether containing the neutral/basic volatiles was dried over anhydrous sodium sulfate. The sodium bicarbonate wash was re-acidified with 18% v/v of 12 N HCl. The wash was extracted with redistilled diethyl ether (3x50 mL). Finally, the ether containing the acidic fraction was dried over anhydrous sodium sulfate. Both ether fractions of the samples were concentrated to approximately 1 mL by distillation with an insulated Vigreux column, followed by a purge with a steady stream of purified nitrogen to a final concentration of 250 µL. Samples were analyzed on a Leco Pegasus IV GC-MS equipped with a HP5 column (60 m x 0.25 mm x 0.25 µm). GCO analysis was conducted with two trained panelists on a HP-6890 GC-MS equipped with a DB-WAX and HP5 column (60 m x 0.25 mm x 0.25 µm).

Quantitation methods were developed for selected off-flavor compounds and compounds characteristic to lipid oxidation present in the milk samples: nonanal, borneol, 2-methylisoborneol, α-terpineol, E-2-decenal, and E,E-2,4-decadienal. Internal and external standard techniques were employed to calculate the quantities of these compounds within each milk sample.

Results and Discussion *(Please write your discussion in past tense and use a format similar to a publication.)*

Comparative GC-O analysis was utilized to characterize differences in the aroma profiles of the SOF milk and the control milk. Compounds that were at higher concentration in the SOF milk were targeted as possible off-flavor compounds identified by GC-MS-TOF (verified with an authentic compound). A list of the main odorants reported in the 14 day old SOF milk sample is reported in Table 1. Several compounds that appeared in the SOF milk sample were not present in the control milk. These compounds included the following terpenoids: borneol, 2-methylsiborneol, and α-terpineol. These odorants are known to have green or musty odors, often at very low concentrations (part-per-billion to part-per-trillion levels). In addition to terpenoids, lipid oxidations products, specifically trans-2-decenal and E,E-2,4-decadienal were also identified in the SOF sample and not reported in the control. The presence of these two particular aldehydes in the SOF milk may point to a more specific oxidative pathway or precursor in the milk, particularly since there was a lack of other typical lipid oxidation markers. Other aldehydes characteristic of lipid oxidation did not show an appreciable difference in concentrations between milk samples.

The quantities of the select terpenoids and lipid oxidation compounds over 14 days of storage are reported in Figures 1 & 2. Over 14 days, borneol and α-terpineol seemed to decrease in concentration, possibly as a result of decomposition or oxidative reactions. However, 2-methylisoborneol appeared to increase in concentration over the 14-day period; this suggested 2-methylisoborneol was generated during the aging process.

Table 1, Flavor profile of SOF milk stored at 5°C after 14 days post-pasteurization. GCO intensities were identified using the following descriptors: s = strong, m/s = medium/strong, m = medium, w/m = weak/medium, w = weak, vw = very weak.

	DB-WAX	HP-5	Odor Descriptor	Intensity	Identified compound
1	717	351	etherial	w/m	acetaldehyde
2	1523	687	pungent, rancid	w/m	propanoic acid
3	-	732	sulfurous, egg	m/s	unknown
4	1107	777	oxidized, fat	w/m, m	hexanal
5	-	816	rancid, onion	w/m	butyric acid
6	1347	844	grass	vw	hexanol
7	1195	882	creamy, fat	w	2-heptanone
8	-	960	slight mushroom	vw	unknown
9	1347	981	mushroom, earthy	m/s	octanol
10	1872	1040	sweet	w	benzyl alcohol
11	1550	1076	pungent	vw	octanol
12	1435	1090	soap, musty	w	2-nonanone
13	1347	1112	fat, cream	m/s	nonanal
14	1657	1169	earth, musty	w/m	borneol
15	1573	1175	musty	w/m	2-methylisoborneol
16	1696	1189	musty, mothball	w/m	naphthalene
17	1678	1192	earth, musty	w/m	alpha-terpineol
18	1550	1197	green, sweet	w/m	decanal
19	1573	1240	fat, wax	w	E-2-decenal
20	1642	1252	oxidized, fat	w	E,E-2,4-decadienal
21	1696	1272	floral	w	decanol
22	2163	1281	coconut	w/m	delta octalactone
23	2075	1344	phenolic, woody	w/m	p-vinyl guaiacol

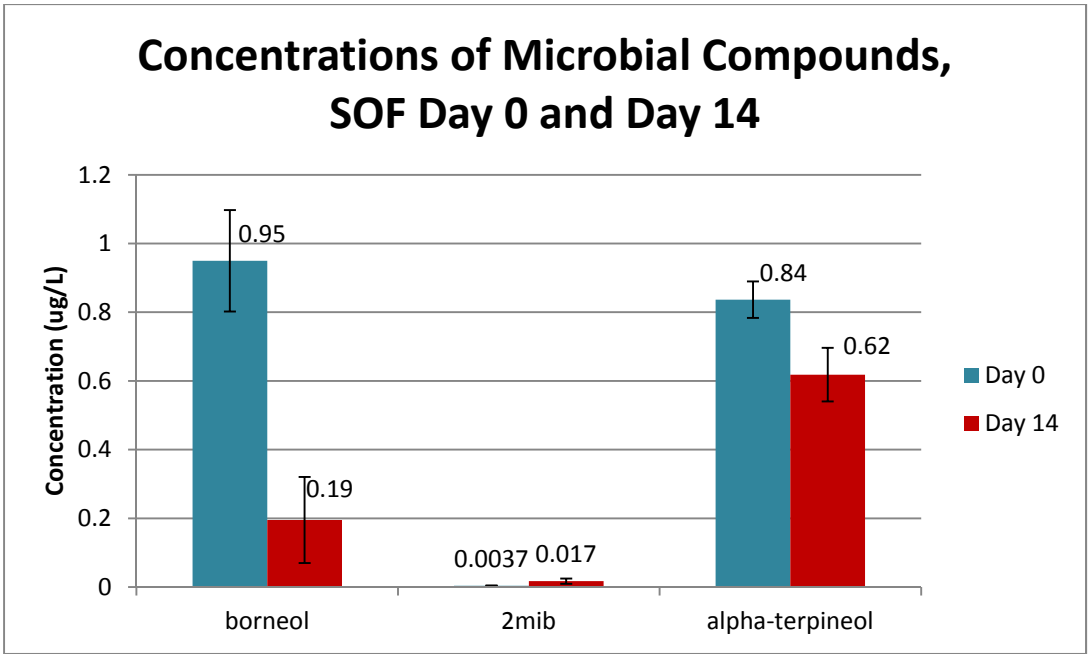

Figure 1 – Concentration of microbial off-flavor compounds reported in objectionable flavored milk sample at day 0 and 14 under refrigerated storage

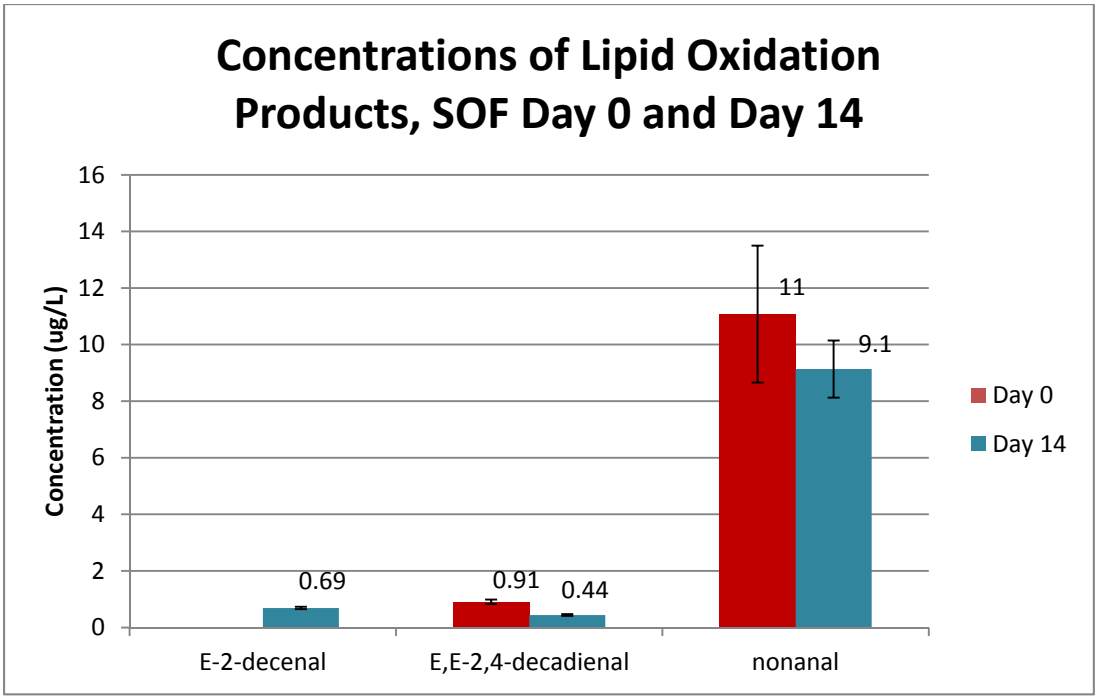

Figure 2 – Concentration of select lipid oxidation off-flavor compounds reported in objectionable flavored milk sample at day 0 and 14 under refrigerated storage

These terpenoids are known to occur by microbial sources and have been connected to drinking water, animal feed, and silage. Considering the origin of these noted off-flavor compounds it is likely a seasonal alteration in one of these sources could result in microbial growth and off-flavor contamination of milk. Some literature sources have also cited particular strains of thermoacidophilic bacteria, such as *Streptomyces* species, as being resistant to pasteurization and causative of similar terpenoids [5]. If such bacteria survived pasteurization, it is possible that they could propagate and

cause further off-flavor development in milk. This might account for the increasing undesirability of the milk over time, as well as its ability to affect otherwise "clean" milk during bulk storage, particularly since these odorants are very potent and can be perceived at extremely low concentrations. This survival of related off-flavor strains in pasteurized milk would also potentially explain why 2-methylisoborenol was reported to increase during storage in our sample (Fig. 1).

An initial sensory study with the compounds at the given concentrations resulted in a similar sensory response to that of the original SOF samples. Milk spiked with the levels of the terpenoid compounds had a slight "green" off-flavor and aftertaste, as well as a slight metallic mouthfeel. A more formal sensory study will be conducted during the month of July to verify whether the levels observed in these milk samples produce a similar sensory response and off-flavor defect in milk. The original SOF samples will be compared with clean milk spiked with comparable concentrations of both the terpenoid compounds and lipid oxidation compounds. This will aid in the determination of the impact that the terpenoids may have individually as well as in combination with the observed lipid oxidation products. Initial analysis of the lipid oxidation products suggested they were not the cause of the off-flavored product.

Conclusions *(Discuss your overall conclusions for your study and provide a 1- or 2-sentence summary for how this study will benefit industry)*

The results obtained from this SOF study point to microbial contamination as the cause of the off-flavor in objectionable flavored milk samples obtained from a farm. Other research has outlined several bacterial sources that could survive pasteurization in juice samples and propagate in processed samples, resulting in increased amounts of these off-flavor compounds during storage similar to those found in this study. Borneol, 2-methylisoborneol, and α-terpineol all appeared to be present within the samples, and initial sensory work suggested that they provide sensory characteristics reminiscent of the samples originally obtained. Future work could include a more extensive sampling of milk from the same farm and the surrounding region, as well as water, feed sources, and microbial assays. A year-round sampling of farm conditions and subsequent flavor analyses of these sources could provide insight into a more specific source of these off-flavors, as well as routes of inhibition.

References *(Please cite any references)*

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