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Research Article

Volatile Components in Breast Milk

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- Volatile components
- Mother's diet
- Retention index
- Flavor and aroma
- Milk samples

Abstract

First experience of an infant with various kinds of flavors in diet is through breast milk where odors and flavors transmit crucial information of the outside world. In this study, volatile components in breast milk affecting its flavor and aroma were analyzed showing the effect of mother's diet on characteristic volatile components of breast milk.

16 samples of breast milk from 1 donor and 1 sample each of Infant formula milk and semi-skimmed milk were tested in a gas chromatograph, which was also analyzed against the respective diets of the mother in order to make an inference about effect of lactating mother's diet on the presence/absence of specific volatile components in milk.

11 common volatile compounds were identified in breast milk, cow's milk and Infant formula milk in different quantities. But when lactating mother was given varying diets, breast milk showed significant increase/decrease in the volatile composition compared to the semi-skimmed and formula milk whose ingredients were kept constant. Breast milk showed a varied composition of these 11 components among each sample.

Breast milk shows comparatively vast flavor and aroma profile than formula milk and cow's milk inferring breast-fed infants are used to a diversity of tastes and odors that might affect their food preferences and openness to different flavors later in life.

INTRODUCTION

Systems/organs of a newborn infant are not fully developed which affects their digestion and absorption of nutrients as well as excretion of metabolites. Hence, their nutrient requirements are very specific, i.e. enough nutrients are needed for proper growth but too much can be harmful [1].

Mother's milk is nutritionally adequate and easily digestible for infants.

Usually cow's milk, (semi-skimmed milk) is used to substitute Breast milk that has some compositional differences, which hinders the growth and development of an infant. Moreover, breast milk makes up for antimicrobial agents and various kinds of flavors for the baby, which is not fulfilled by cow's milk.

Bottle-feeding with formula milk is a convenient way of feeding infants. The composition of formula milks available in market are governed by respective food agencies, like: In India its FSSAI and in Britain, it is governed by a directive from the European commission (EEC, 1991), and statutory instrument 77 (MAFF, 1995) [1].

Breast milk has the unique composition containing more than 200 constituents with a needed concentration which a formula feed can never match [2]. The composition of the breast milk keeps on changing not only during a day but also while feeding and is never constant. This provides both energy and nutrient dense food to infant which rarely happens with formula feed.

As there is a substantial difference in flavor and aroma preferences of an individual giving rise to various dietary habits and food choices among human beings. It is believed that the milestone for these flavor and aroma perceptions is decided during child's early flavor experiences with food which can be in form of mother's breast milk or an infant formula milk. The dietary flavor compounds of the mother's diet are transferred in to breast milk and hence, chemical and sensory composition of breast milk constantly changes. These compounds mostly manifest as volatile compounds in milk.

Nevertheless, during infancy, child is more receptive to sensory and cognitive learning and behaviors established in this period are most probably important for their later preferences and food behaviors. This is because lactating mother always consumes different kinds of foods and beverages that influence the taste and flavor of breast milk. This gives an infant various taste experiences making them open to different flavors and aromas.

On the other hand, formula milk or semi- skimmed milk has constant flavor and aroma all time with same components. However, constitution of formula milk can be changed by the industries from time to time to meet the need of the infants or maybe just to change flavor and aroma of the milk, which affects its volatile components. Even then, child gets used to a particular type of taste and aroma due to lack of change.

Hence, in addition to all nutritional benefits of breast milk, breast milk is important to influence eating behavior of an individual as there are some evidences to show that the breast fed infants subsequently adopt more diverse diet due to diversity of flavors and aromas introduced in infant's diet through mother's diet [3].

Therefore, to check whether changes in diet affects volatile constituents of breast milk changing its taste and aroma profile, and to mark a difference in volatile components present in formula milk, breast milk and cow's milk, following experiment was performed.

Transfer of flavor through breast milk

During fetal stage, flavor and aroma experience is through amniotic fluid. This experience is taken to next level during infancy through breast-feeding. Many substances from mother's diet filters in breast milk giving it a particular flavor and aroma providing cues to the infants.

For instance, Garlic ingestion by the mother increased suckling and length of feeding with an overall increase in consumption of cereals [4]. This also demonstrates the lure of mammals to some chemicals containing sulphur. This was also observed in vanilla study and alcohol study with vanilla alluring the innate liking for sweetness [5] and alcohol probably having a more neurological effect, maybe relaxing, than the initial sensory appeal.

However, during these studies, none of breast milk was actually checked for presence of flavor compounds.

Transfer of various flavors through breast milk over time

A more inclusive study examined a diversity of typical aroma compounds to determine their disparity transfer into breast milk and it's time reliance. It was found that greatest concentrations for d-carvone and trans-anethole were observed two hours post intake whereas l-menthol showed no distinction in concentration over time [6]. Some compounds such as ester-3-methyl butyl acetate were not traced at all perhaps showing that breast milk synthesis mechanisms are discriminating in their utilization of the mother's diet components. Otherwise, 3-methyl butyl acetate could be too large a molecule to circulate into the breast milk or its chemical composition is changed in body and therefore, is not detected in milk. Overall, it was inferred that taste compounds do transfer specifically from mother's diet to her breast milk.

Transfer of volatile components to breast milk

Various factors affect the concentration of volatile components in breast milk including mother's diet and modes of transmission. Various ways of transfer of volatile components to breast milk are:

Metabolism in mother's body

So far, there has been a little delving into the field of human metabolism of specific flavoring compounds. Generally, the research studies available are based on terpene content in humans during drug exposure or in animal analogue studies. Although animal studies suggest some practical theories on the outcome of terpenes [7], but it is still a topic of confusion whether these studies can be used to infer anything about the human body.

Additional researches were conducted to find the intake of terpenes through drug exposure versus its presence in diet. It was found that carvone (found in most essential oils, therefore widely used in food flavoring and cosmetics) was involved in oxidation and reduction reactions in body altering its chemical composition to form new products [8].

Absorption of volatile compounds in breast milk using a carrier

Studies show that fats act as a 'carrier' of volatile compounds [8]. There will be more fat molecules circulating in mother's blood if her diet is high in fat. This increase in fat components in blood increases its chances of flavoring components to appear in the breast milk.

Transportation across epithelial cell membranes

Mother's diet not only enhances the volatile profile of breast milk but it can also taint it. Transfer of flavor compounds, drugs and chemicals is likely to be mediated by the same transfer and secretary pathways as milk solutes, i.e. trans-cellular pathway through passive diffusion [9]. In studies where the dose of a flavor compound has been measured to mimic the dose and release of drugs during digestion, milk and plasma peaks were found to correspond to those of drugs [6]. Hence, passive diffusion is the way through which flavor compounds are transported from blood in milk. Usually, if drugs have low plasma protein binding, high lipophilicity, positive charge and low molecular weight, then excretion are facilitated. Milk is lower in pH and higher in fat content as compared to blood plasma; therefore, this enhances the passive transmission through diffusion towards higher concentrations in milk than the blood [10].

MATERIALS

Breast milk samples

Sixteen breast milk samples were obtained from one donor. A healthy lactating women aged 35 years approx. of European origin was selected as a donor for breast milk samples. (An informed consent was taken from the donor for the use of milk for this study.) Samples were collected from the donor in between the main meals.

Each sample was approximately 5 - 10ml in volume for experimental use, and stored into sterilized containers which were then frozen at -72°C.

Corresponding diet details were provided with respective breast milk samples and were categorized in accordance with taste - alcohol consumption (alcohol/no alcohol) and smoking/ no smoking. Each breast milk sample was given a number corresponding to the dietary behaviors for that sample and also depending upon the source breast (left/ right).

Formula milk samples

Only one type of formula milk was used in this experiment - liquid infant formula milk. It was from a well-known infant formula milk brand, purchased in the UK. The milk came in a 200ml ready to use recyclable carton that is a popular brand of formula milk stocked throughout the UK providing a wide range of nutritionally adequate baby milks for different ages with 'from birth onwards' being selected based on its specific flavor and nutritional content made from cereals, fruits, vegetables, etc. The contents used in manufacturing the milk can affect its volatile composition and flavors.

However, Infant formula has skimmed or semi skimmed milk as a base product, but it also has other ingredients in order to increase its nutritional value or enhance its flavor.

Ingredients of the formula milk used in the study

Demineralised Water, Skimmed **Milk**, Lactose (from **Milk**), Vegetable Oils (Palm Oil, Rapeseed Oil, Coconut Oil, Sunflower Oil, Single Cell Oil) (contains **Soy** Lecithin), Galacto-Oligosaccharides (GOS) (from **Milk**), Whey Protein Concentrate (from **Milk**), Emulsifier (Mono- & Diglycerides of Fatty Acids), Fructo-Oligosaccharides (FOS), Acidity Regulator (Citric Acid), Calcium Phosphate, Vitamin C, Potassium Chloride, **Fish** Oil, Calcium Hydroxide, Potassium Bicarbonate, Potassium Citrate, Potassium Phosphate, Choline Chloride, Sodium Chloride, Potassium Hydroxide, Taurine, Magnesium Oxide, Inositol, Iron Lactate, Vitamin E, Zinc Sulphate, Uridine 5'-Monophosphate Disodium Salt, Vitamin A, Cytidine 5'-Monophosphate, L-Carnitine, Adesonine 5'-Monophosphate, Inosine 5'-Monophosphate Disodium Salt, Vitamin D3, Niacin, Pantothenic Acid, Guanosine 5'-Monophosphate Disodium Salt, Copper Gluconate, Sodium Selenite, Potassium Iodide, Folic Acid, Biotin, Riboflavin, Vitamin B12, Vitamin K1, Thiamin, Vitamin B6, Manganese Sulphate.

(Source: Formula milk carton used)

Cow's milk samples

Semi skimmed milk sample was used for the experiment which was bought locally and was stored in a sterilized container in the deep freezer at -72°C.

Carbon Standards

The carbon standards used in the experiment were obtained from the chemistry laboratory of University of Huddersfield, UK.

Gas Chromatograph

The technique available in the laboratory of University of Huddersfield, which was used to perform the experiment.

METHOD

Gas Chromatogram

Ease of use and practicality was a factor for choosing this method. The aim of the experiment was to identify the volatile components that affect the aroma and flavors of the milk. Different samples of milk were tested under different conditions

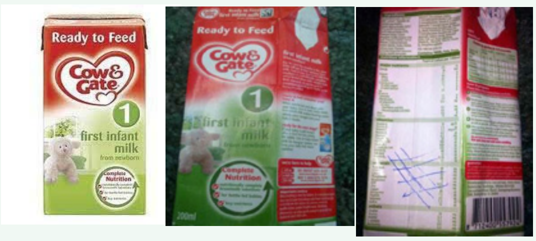

Figure 1 Pictures of the formula milk used for the study with details of the preparation method, ingredients and nutritional content.

and respective volatile compounds in the milk were analyzed from the results obtained. The instrument used in testing the samples was a gas chromatograph [11].

Model of instrument used: 25QC3 BPX5 0.5.

Serial number of model: 3553517.

Length of the column: 25n

Thickness of the film: 0.5pm

Maximum temperature: 350°C

A trial was made to ensure instrument was working in a proper manner. Trial was carried out using five samples. Samples were:

1. Carbon standard ($C_8 - C_9 - C_{10}$)
2. 0 - xylene
3. m - xylene
4. p - xylene, and
5. Unknown compound, that was to be identified.

Different samples were kept inside machine, and retention time for the samples was noted. It was observed, that the retention time for every sample was obtained in 10 minutes. By comparing retention times of different samples, unknown compound was identified.

Second trial was carried out by using methane, where samples of methane were taken in different proportions. Methanol was converted to methane by water distillation method and then it was used for the experiment. Three different types of syringes were which were of 1ml, 20ul, and 10ul. Different amounts of methane were used, and corresponding retention time was noted.

Main experiment was carried out by using formula milk, breast milk and the cow's milk while considering different parameters like time of the sample in the water bath, apparatus parameters, and the amount of sample tested.

Experiment was first carried out by testing formula milk.

10 ml of the sample was taken using a pipette and was transferred to a test tube. Then it was placed in a water bath for 30 minutes, which was maintained at 37°C. The test tube was fitted with a rubber cork, so the gases released from the samples are held tight inside the test-tube. 1ml of the volatile component (gas) is taken using an injection and was then injected in the gas chromatograph.

The sample was kept inside the machine with default settings for around 20-30 minutes, because different samples contain different Boiling Point and the peak times also differ according to the samples. With this experiment carried out, no peak was observed, so the test was repeated by changing the experimental conditions as under:

Formula 1

Experiment was repeated by setting an oven temperature to 70°C, the flame ionization detector used was set to 280°C, injector temperature to 240°C, split ratio 50:1, and flow rate 1ml/min. No peaks were observed again, so the experiment was repeated

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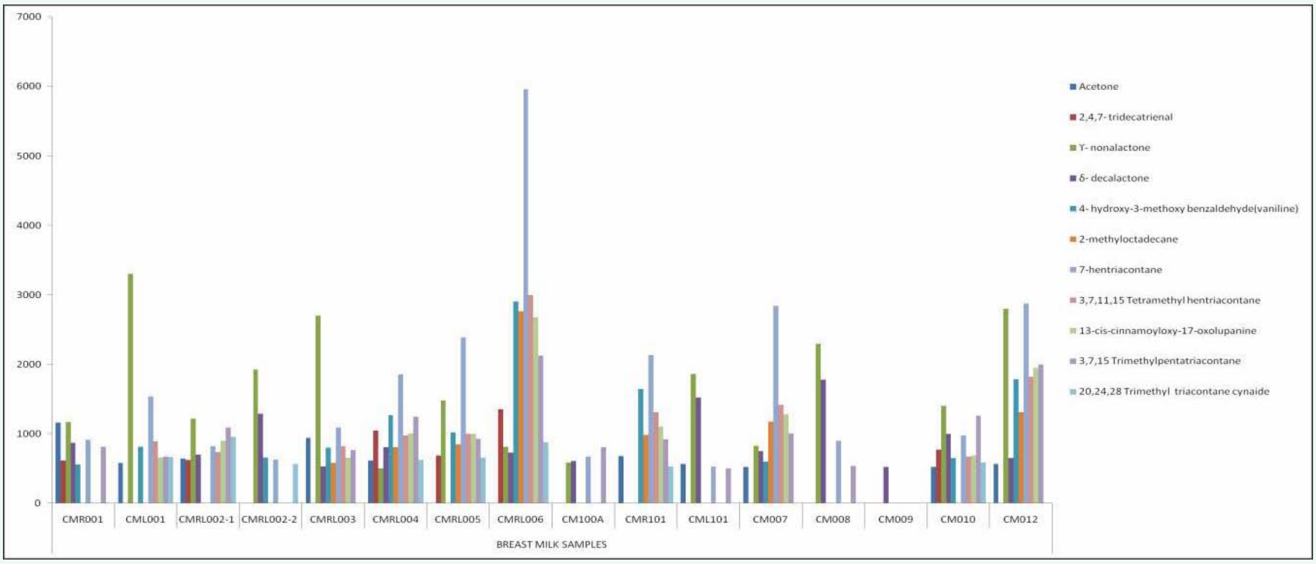

Figure 2 In conjugation with (Table 3). [All the eleven volatile compounds found to be present in all breast milk samples tested in gas chromatograph are listed with their peak areas respectively. It can be seen that many samples show varied number of peaks. This can be due to the effect of the diet on the milk.]

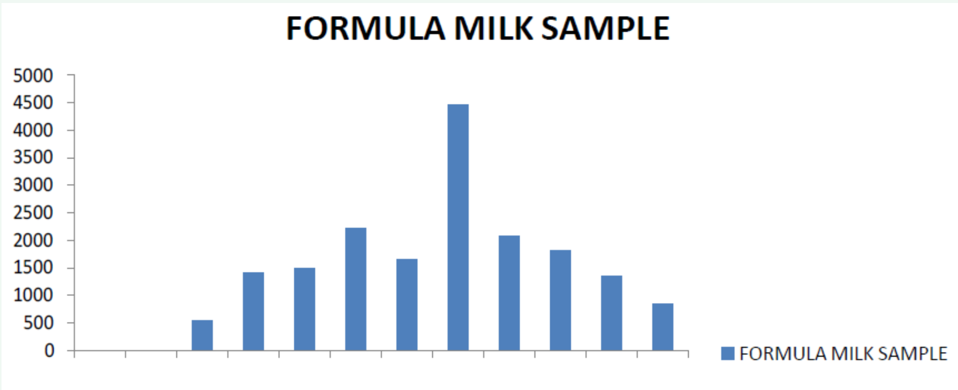

Figure 3 In conjugation with (Table 4). [Peak areas of Volatile components found in formula milk sample upon testing in the gas chromatograph. Comparing the major volatile compounds of formula milk with the breast milk, it can be seen that acetone is the only compound which is absent in the formula milk which has got a sweet and fruity flavor.]

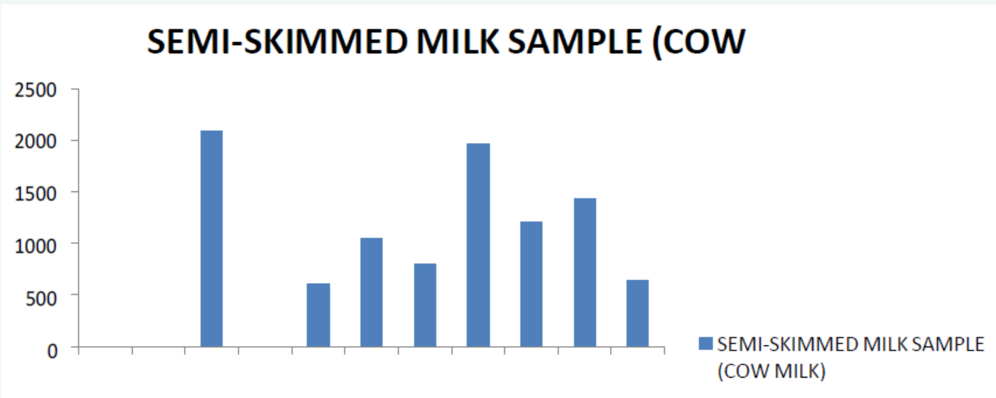

Figure 4 In conjugation with (Table 5) [Peak areas of Volatile components found in Cow milk sample upon testing in the gas chromatograph. When the peak areas of semi- skimmed milk is compared with the breast milk it can be seen that Acetone, 2,4,7 tridecatrienal, and decalactone are absent]

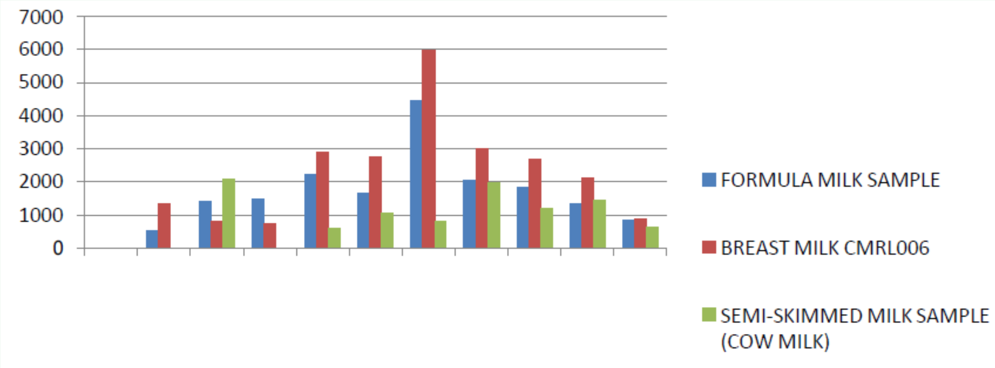

Figure 5 In conjugation with (Table 6). [Comparison of three types of milk (breast milk CMRL006). amount of compounds present in the breast milk is much higher than the other milks].

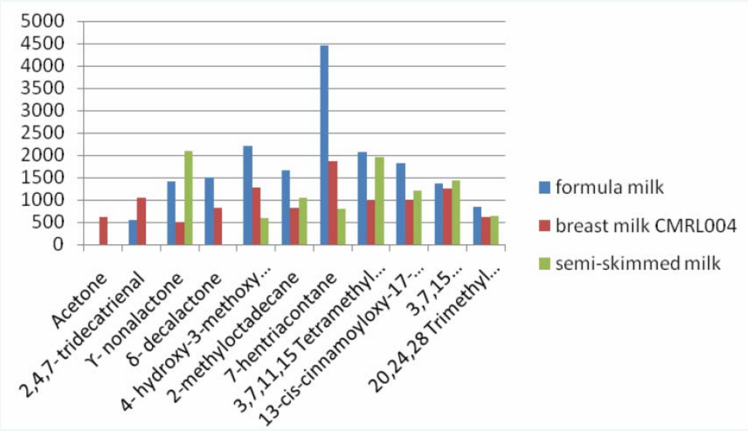

Figure 6 In conjugation with (Table 7). [Comparison of three types of milk (breast milk CMRL004). The graph above shows that formula milk's skyline dominating the rest.]

again by changing the parameters.

Formula 1 SL

Experiment was repeated using same parameters used in formula 1 but by using split-less injection method. Three samples were tested which were kept in water bath for different time intervals say 1, 2 and 3 hours with a run time of 30 minutes. Again, no major peaks are obtained.

Formula 2

In this case, 2 samples from the same formula milk were taken (Sample 1: 15ml and Sample 2: 10 ml), the experiment was carried out using split-less method by setting the parameters as listed below,

Flow rate: 2.2 ml/min.

Oven temperature: 40 to 250°C, where temperature was increased by 6 degree every minute for 35 minutes, and was held at 250 degrees for 10 minutes, therefore making it a total run time of 45 minutes [12].

This time, there was a difference in readings, where sample tube 2 showed more peaks compared to the sample tube 1. The reason for difference in the peaks could be due to the fact that sample tube 1 didn't have a safety clip, and the gases could have escaped through the hole in the cork. Ultimately some volatile components might have be lost in the air resulting in lesser peaks.

Other reason for difference could be the amount of milk and length of time for which milk has been kept in water bath may affect amount of volatile components released. Shape of the container might also affect the release, where a wide container may favor it due to more space for molecules compared to a narrow container.

Results obtained with 10ml formula milk sample had higher peaks compared to the 15ml sample. With an Idea that lower amount of sample will give higher amount of volatile components, the experiment was carried out by reducing the amount of samples to 5ml, 2ml and 1ml.

From experiments, it was observed that best results were obtained from 1ml sample. So, test was continued using the amount of 1ml with different samples under formula 2 settings.

Breast milk samples were taken from deep freezer, were defrosted for 45 minutes in the water bath maintained at 37°C. From defrosted milk, 1ml sample was taken from quick fix test tube, and was kept in the same water bath for one hour and experiment was continued as discussed above.

Sixteen different samples were tested, and testing was repeated for all the samples over time to ensure if sample components changed over time and the results were compiled.

Before carrying out test, septum in apparatus was changed and a blank run was done using methanol for cleansing stains in apparatus. Experiment was repeated using same settings as used in *formula 2*.

Sampling of carbon standards C_{10}-C_{40} were also done with the same settings and same amount of sample in order to find out their retention times (Figure F).

Calculation

Retention time for every sample was noted.

Kovat's retention Index was calculated using the formula shown below

$$X = \frac{\log CRT + 3.897}{0.0045}$$

Where CRT = RT – 1.371

And, log (CRT) = 0.0045x – 3.897

Where RT – Retention time, X – Retention index

Retention indices obtained by using above formula are for isothermal system. As the above testing involved increasing temperature at constant rate, this formula could not be used; instead a non-isothermal retention index formula had to be used.

Kovat's Retention Index for non-isothermal system is:

$$RI = 100\ [\ {T_rC_n - T_rC_{n-1}} \Big/ {T_rC_{n+1} - T_rC_{n-1}}\ \ C_{n-1}\ +]$$

Where RI = Rvvetention Index,

$T_r\ C_n$ – Retention Time of the unknown compound,

$T_r\ C_{n-1}$ – Retention time of standard carbon compound with one less carbon atom than unknown (X)

$T_r\ C_{n+1}$ - Retention time of standard carbon compound with one more carbon atom than unknown

C_{n-1} - Number of carbon atoms in (X)

Above formula was used in calculating retention indices of the obtained peaks of eleven major volatile compounds identified in milk samples tested, and it was also used for calculating RI of carbon standards.

The retention time of a compound peak in the breast milk was taken as the average of all the major peaks obtained in sixteen samples at that time showing the presence of same compound. Now, the retention time for peak of the breast milk was compared with the carbon standards to find the ones with similar retention time. The retention time and the retention index of different carbon standards are shown in the Table (1).

Though numerous peaks were obtained during the test, only few major peaks were noted where the first peak is obtained at the 4th minute and the last higher peak considered was the one obtained at 37th minute. There were some peaks obtained below the retention time of 4 and above the retention time of 37, but the carbon standards available had their retention times starting at 4 till the maximum of 39. Therefore, we could calculate in this range only.

Eleven major peaks were obtained in all the samples of the breast milk and the retention times for peaks were noted. (Sample of peaks and retention times obtained in gas chromatograph for breast milk and semi skimmed milk attached in Figures (G-P)).

Then the retention time and Index of the particular carbon standards were used along with the retention time of a major peak of the breast milk in the formula -

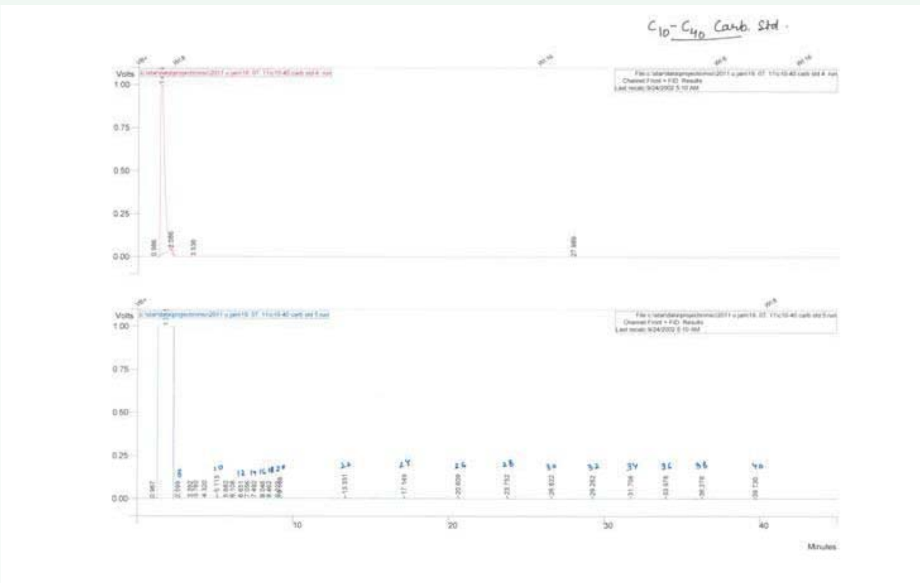

Figure 7 Carbon standards run in gas chromatograph to obtain retention peaks and times.

Table 1: [Calculation of retention indices of carbon standards].

Number of Carbon Atoms	Retention Time	Retention Index
8	2,658	800
10	5,139	865
12	6.491	1075
14	6,946	1232
16	7,887	1461
18	8,493	1646
20	9,181	1841
22	13,334	2052
24	17,156	2252
26	20,614	2452
28	23,757	2651
30	26,627	2850
32	29,267	3051
34	31,709	3256
36	33,977	3449
38	36,277	3640
40	39,727	3860

SPSS ANALYSIS
Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Alcohol	9	6.78	4.684	1	13
Smoking	11	6.64	3.749	1	12
Yoghurt	7	7.29	4.821	1	14
Tea	7	7.14	4.337	1	12

Descriptive Statistics.

$$RI = 100\left[\frac{T_rC_n - T_rC_{n-1}}{T_rC_{n+1} - T_rC_{n-1}} + C_{n-1}\right]$$

Hence, the corresponding retention index of breast milk's peak is calculated.

For example, the retention time of one of the major peak in breast milk was 4.279 (From sample test results). This was compared with the retention time of the different carbon standards, and it can be seen that 4.279 lies in between the retention time of the carbon standards C8 and C10, which were 2.658 and 5.139 respectively. In the formula, C_n: 4.279, C_{n-1}: 2.658, C_{n+1}: 5.139

By substituting the values, it can be seen that the Retention index of the unknown peak is 862. Using the retention index, the unknown compound was identified as acetone.

The same calculation was done for all the eleven major peaks and the retention index for all was found which can be seen in the Table (2).

RESULTS

Different milk samples were tested using gas chromatography technique for assessing volatile components that affects its flavor and aroma, which in turn helps in understanding the effect of mother's diet on composition of breast milk.

Various peaks were obtained for each sample at different time intervals, i.e. retention times.

The retention time is the time at which a peak is obtained.

The eleven major volatile compounds found in the breast milk were:

Acetone
2,4,7 tridecatienal
ϒ- nonalactone
δ decalactone
4- hydroxyl-3-methoxy benzaldehyde (vaniline)
2- methyloctadecane
7 hentriacontane
3,7,11,15 tetramethyl hentriacontane
13-cis-cinnamolyloxy 17- oxolypanine
3,7,15 trimethyl pentatriacontane
20,24,28 trimethyl tritriacontyl cyanide.

The effect of diet on the volatile components in the breast milk can be seen with the help of (Table 2,3) along with the graph

Table 2: [Shows the volatile components found in the milk with their retention indices and characteristic flavor and aroma].

S.No.	Volatile Compound Found	Retention Index	Flavor and Aroma
1	Acetone	862	Fruity Sweet
2	2,4,7- tridecantrienal	1491	Pig, animal, bitter sweet, citrus peel waxy fatty oily fruity
3	γ – nonalactone	2037	Fruity dairy orchid
4	δ - decalactone	2219	Intense peach flavor, creamy coconut aroma
5	4-hydroxy-3-methoxy benzaldehyde (vaniline)	2633	Vanilla
6	2- methyloctadecane	2870	Citrus, rose
7	7- hentriacontane	3074	Tobacco like
8	3,7,11,15 Tetramethyl hentriacontane	3257	Fatty acid taste and odor
9	13-cis-cinnamoxy-loxy-17- oxolupanine	3417	Sweet and salty
10	3,7,15 Trimethylpenta-triacontane	3638	Sour
11	20,24,28 Trimethyl tri-acontane cynaide	3841	Bitter, almond like odor

Test Statistics

	alcohol	smoking	yoghurt	tea
Asymp. Sig.	1.000	1.000	1.000	1.000

Table 2 Test statistics. Above tables were obtained by the statistical analysis of the diet for effect of alcohol, smoking, yoghurt and tea. Chi- square test was performed to check their significant on the milk compounds. It shows that all these diet constituents have a significant effect on the volatile components of milk. As the p- value (asymp. Sig.) of all the diet constituents is 1.000 which is more than 0.05. Hence, concluding that there is very less evidence to show that there is no effect of alcohol, smoking, yoghurt and tea on the volatile composition of the breast milk

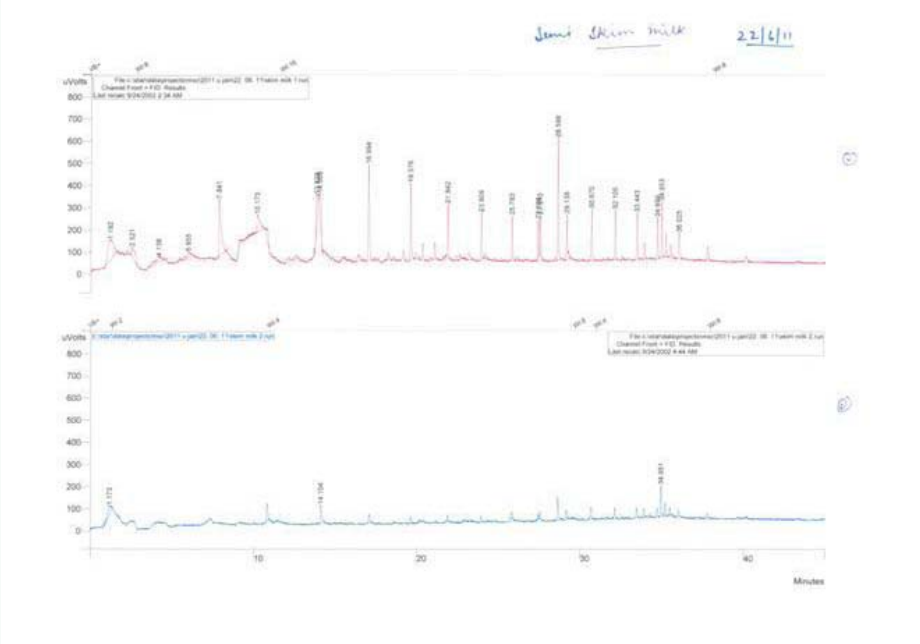

Figure 8 Peaks obtained in gas chromatograph for Semi skimmed milk sample.

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Print Date: Tue Sep 24 00:11:42 2002            Page 1 of 1

Title       :
Run File    : c:\star\data\projects\msc\2011 u jain\1. 07. 11\semi skim milk 1.ru
Method File : c:\docume~1\admini~1\locals~1\temp\~semi skim milk 1-front.tmp
Sample ID   : semi skim milk 1

Injection Date: 9/24/2002 1:35 AM     Calculation Date: 9/24/2002 4:42 AM

Operator    : Richard                 Detector Type: 39XL (1 Volt)
Workstation:                          Bus Address  : 44
Instrument  : GC CP3900               Sample Rate  : 20.00 Hz
Channel     : Front = FID             Run Time     : 45.000 min

** Star Chromatography Workstation Version 6.00 ** 02467-6490-c65-01e1 **

Run Mode         : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
```

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		10.5912	1.162	0.000	3198	BB	47.3	
2		2.4085	5.810	0.000	727	BB	18.8	
3		6.9125	7.824	0.000	2087	BB	12.5	
4		11.6551	9.959	0.000	3519	BB	13.6	
5		3.9442	13.491	0.000	1191	BB	5.7	
6		2.0870	13.758	0.000	630	BB	8.2	
7		1.7869	15.326	0.000	539	BB	11.3	
8		7.1453	16.940	0.000	2157	BB	5.4	
9		1.7803	18.024	0.000	538	BB	7.7	
10		4.2661	19.523	0.000	1288	BB	3.8	
11		3.4858	21.791	0.000	1052	BB	3.7	
12		2.5744	23.859	0.000	777	BB	3.8	
13		2.6547	25.735	0.000	802	BB	3.9	
14		1.7568	27.332	0.000	530	BB	3.5	
15		2.2544	27.463	0.000	681	BB	4.1	
16		6.5017	28.543	0.000	1963	BB	3.3	
17		3.1727	29.091	0.000	958	BB	4.2	
18		3.9903	30.622	0.000	1205	BB	4.2	
19		3.6437	32.058	0.000	1100	BB	4.1	
20		3.0969	33.399	0.000	935	BB	4.1	
21		2.8581	34.655	0.000	863	BB	4.2	
22		4.7625	34.902	0.000	1438	BB	3.5	
23		2.0173	35.157	0.000	609	BB	4.1	
24		2.5289	35.967	0.000	764	BB	5.7	
25		2.1249	37.690	0.000	642	BB	6.7	
	Totals:	100.0002		0.000	30193			

```
Total Unidentified Counts :       30192 counts

Detected Peaks: 116          Rejected Peaks: 91       Identified Peaks: 0

Multiplier: 1             Divisor: 1           Unidentified Peak Factor: 0

Baseline Offset: -11 microVolts            LSB:     1 microVolts

Noise (used): 2 microVolts - monitored before this run

Manual injection

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```

Figure 9 Retention times obtained in gas chromatograph for Semi skimmed milk sample.

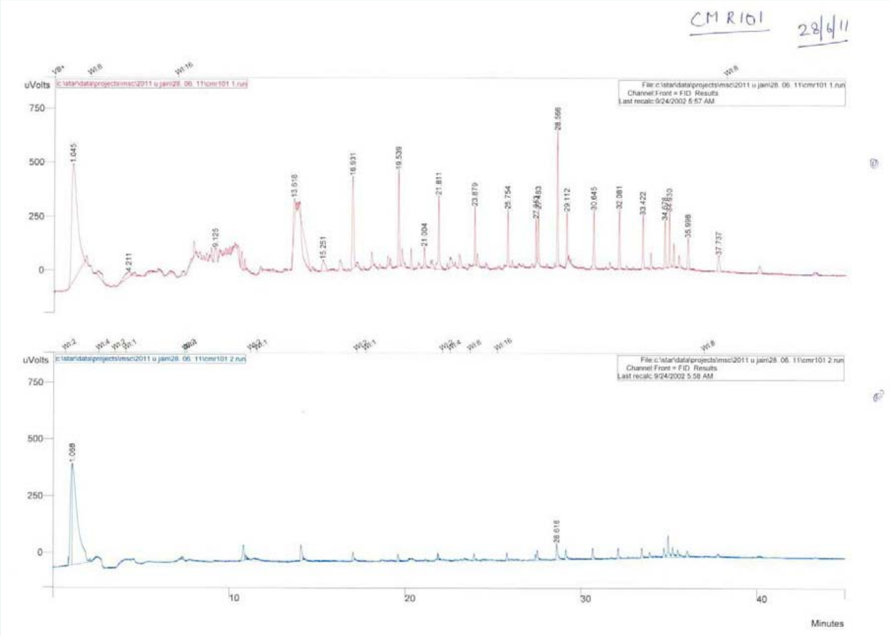

Figure 10 Peaks obtained in gas chromatograph for Breast milk sample CMR101.

28/6

Print Date: Tue Sep 24 00:40:21 2002 Page 1 of 1

Title :
Run File : c:\star\data\projects\msc\2011 u jain\28. 06. 11\cmr101 1.run
Method File : c:\docume~1\admini~1\locals~1\temp\~cmr101 1-front.tmp
Sample ID : CMR101 1

Injection Date: 9/24/2002 2:14 AM Calculation Date: 9/24/2002 5:57 AM

Operator : Richard
Workstation:
Instrument : GC CP3900
Channel : Front = FID

Detector Type: 39XL (1 Volt)
Bus Address : 44
Sample Rate : 20.00 Hz
Run Time : 44.996 min

** Star Chromatography Workstation Version 6.00 ** 02467-6490-c65-01e1 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		36.0907	1.045	0.000	12531	BB	24.3	
2		1.9483	4.211	0.000	676	BB	0.0	
3		1.6752	9.125	0.000	582	BB	0.0	
4		2.3093	13.618	0.000	802	BB	10.5	
5		1.8312	15.251	0.000	636	BB	16.5	
6		8.0283	16.931	0.000	2788	BB	6.5	
7		4.8498	19.539	0.000	1684	BB	3.7	
8		1.4851	21.004	0.000	516	BB	5.8	
9		4.7388	21.811	0.000	1645	BB	3.8	
10		2.7562	23.879	0.000	957	BB	3.8	
11		2.8356	25.754	0.000	985	BB	3.9	
12		1.7820	27.353	0.000	619	BB	3.7	
13		2.4121	27.483	0.000	838	BB	4.1	
14		6.1608	28.566	0.000	2139	BB	3.3	
15		2.2447	29.112	0.000	779	BB	4.1	
16		3.7723	30.645	0.000	1310	BB	4.3	
17		3.1761	32.081	0.000	1103	BB	4.1	
18		2.8997	33.422	0.000	1007	BB	4.3	
19		2.6160	34.678	0.000	908	BB	4.4	
20		2.6510	34.930	0.000	920	BB	3.7	
21		2.2105	35.998	0.000	768	BB	6.0	
22		1.5263	37.737	0.000	530	BB	6.7	
	Totals:	100.0000		0.000	34723			

Total Unidentified Counts : 34722 counts

Detected Peaks: 126 Rejected Peaks: 104 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: -99 microVolts LSB: 1 microVolts

Noise (used): 3 microVolts - monitored before this run

Manual injection

****

Figure 11 Retention times obtained in gas chromatograph for Breast milk sample CMR101.

SciMedCentral

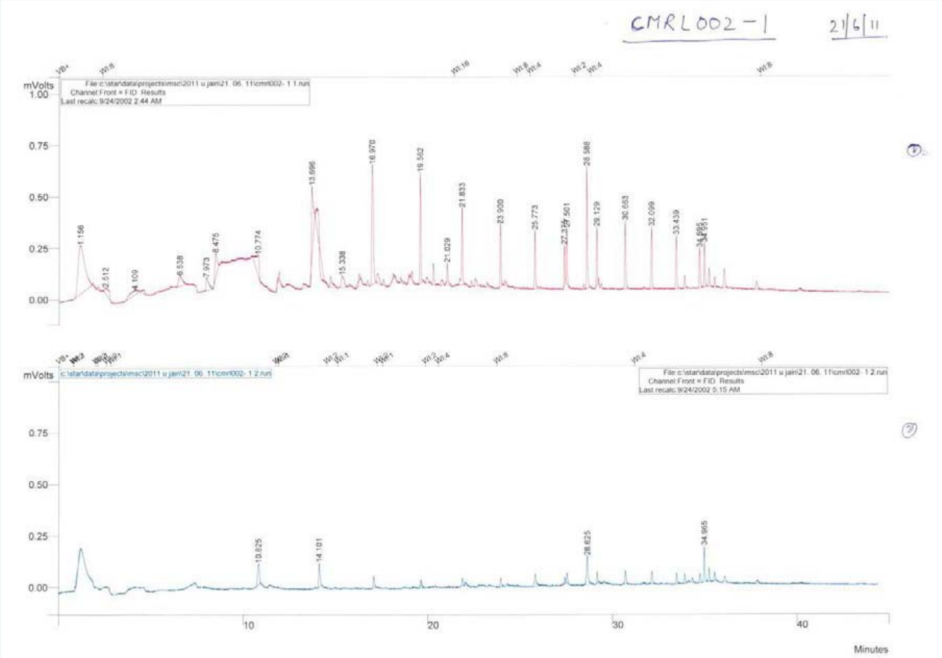

Figure 12 Peaks obtained in gas chromatograph for Breast milk sample CMRL002-1.

Print Date: Tue Sep 24 00:14:56 2002 Page 1 of 1

Title :
Run File : c:\star\data\projects\msc\2011 u jain\12. 07. 11\cmrl002- 1 2.run
Method File : C:\star\Methods\Projects\MSc\2011 U Jain\Formula 2.mth
Sample ID : CMRL002- 1 2

Injection Date: 9/24/2002 3:16 AM Calculation Date: 9/24/2002 4:01 AM

Operator : Richard
Workstation:
Instrument : GC CP3900
Channel : Front = FID

Detector Type: 39XL (1 Volt)
Bus Address : 44
Sample Rate : 20.00 Hz
Run Time : 44.992 min

** Star Chromatography Workstation Version 6.00 ** 02467-6490-c65-01e1 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		27.5449	1.131	0.000	6642	VV	27.5	
2		2.6810	4.000	0.000	647	BV	0.0	
3		2.5752	7.286	0.000	621	BV	29.0	
4		5.0427	10.793	0.000	1216	VV	7.7	
5		2.0892	11.328	0.000	504	VP	30.7	
6		2.8934	14.081	0.000	698	VV	5.4	
7		2.4508	27.483	0.000	591	VV	5.8	
8		3.3952	28.588	0.000	819	VV	5.0	
9		3.2466	29.126	0.000	783	VV	5.7	
10		3.0523	30.649	0.000	736	VV	5.4	
11		3.7252	32.081	0.000	898	VV	5.2	
12		4.8813	33.421	0.000	1177	VV	5.1	
13		3.1806	34.165	0.000	767	VV	0.0	
14		4.5318	34.677	0.000	1093	VV	5.2	
15		5.8674	34.925	0.000	1415	VV	4.3	
16		3.7921	35.182	0.000	914	VV	5.7	
17		5.0637	35.472	0.000	1221	VV	9.3	
18		6.9002	35.991	0.000	1664	VV	7.5	
19		3.1370	37.073	0.000	756	VV	0.0	
20		3.9495	37.726	0.000	952	VV	12.0	
	Totals:	100.0001		0.000	24114			

Total Unidentified Counts : 24115 counts

Detected Peaks: 86 Rejected Peaks: 66 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: -55 microVolts LSB: 1 microVolts

Noise (used): 1 microVolts - monitored before this run

Manual injection

****

Figure 13 Retention times obtained in gas chromatograph for Breast milk sample CMRL002-1.

SciMedCentral

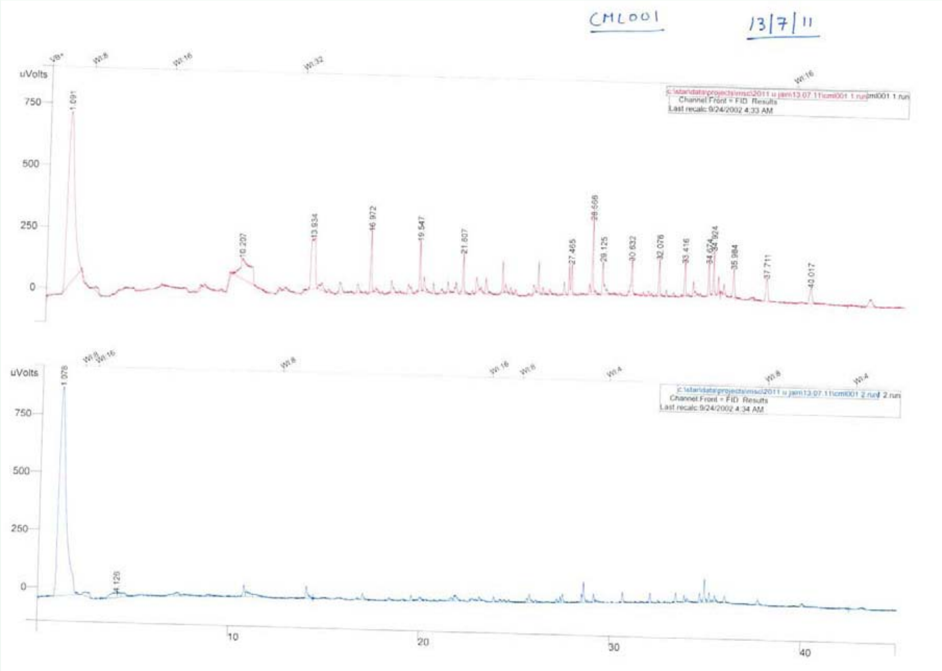

Figure 14 Peaks obtained in gas chromatograph for Breast milk sample CML001.

13/7

Print Date: Tue Sep 24 00:17:17 2002 Page 1 of 1

Title :
Run File : c:\star\data\projects\msc\2011 u jain\13.07.11\cml001 1.run
Method File : c:\docume~1\admini~1\locals~1\temp\~cml001 1-front.tmp
Sample ID : CML001 1

Injection Date: 9/24/2002 1:13 AM Calculation Date: 9/24/2002 4:33 AM

Operator : Richard
Workstation:
Instrument : GC CP3900
Channel : Front = FID

Detector Type: 39XL (1 Volt)
Bus Address : 44
Sample Rate : 20.00 Hz
Run Time : 44.995 min

** Star Chromatography Workstation Version 6.00 ** 02467-6490-c65-01e1 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		48.5143	1.091	0.000	17040	BB	24.0	
2		9.4022	10.207	0.000	3302	BB	68.9	
3		9.0905	13.934	0.000	3193	BB	15.9	
4		3.4959	16.972	0.000	1228	BB	4.6	
5		2.4196	19.547	0.000	850	BB	4.0	
6		2.3102	21.807	0.000	811	BB	4.3	
7		2.6432	27.465	0.000	928	BB	4.8	
8		4.3724	28.566	0.000	1536	BB	3.9	
9		2.4509	29.125	0.000	861	BB	5.3	
10		2.5435	30.632	0.000	893	BB	5.5	
11		1.8715	32.076	0.000	657	BB	4.7	
12		1.7823	33.416	0.000	626	BB	4.9	
13		1.7121	34.674	0.000	601	BB	5.4	
14		1.9153	34.924	0.000	673	BB	4.3	
15		1.9338	35.984	0.000	679	BB	6.4	
16		1.8946	37.711	0.000	665	BB	8.1	
17		1.6477	40.017	0.000	579	BB	9.1	
	Totals:	100.0000		0.000	35122			

Total Unidentified Counts : 35124 counts

Detected Peaks: 77 Rejected Peaks: 60 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: -30 microVolts LSB: 1 microVolts

Noise (used): 3 microVolts - monitored before this run

Manual injection

****

Figure 15 Retention times obtained in gas chromatograph for Breast milk sample CML001.

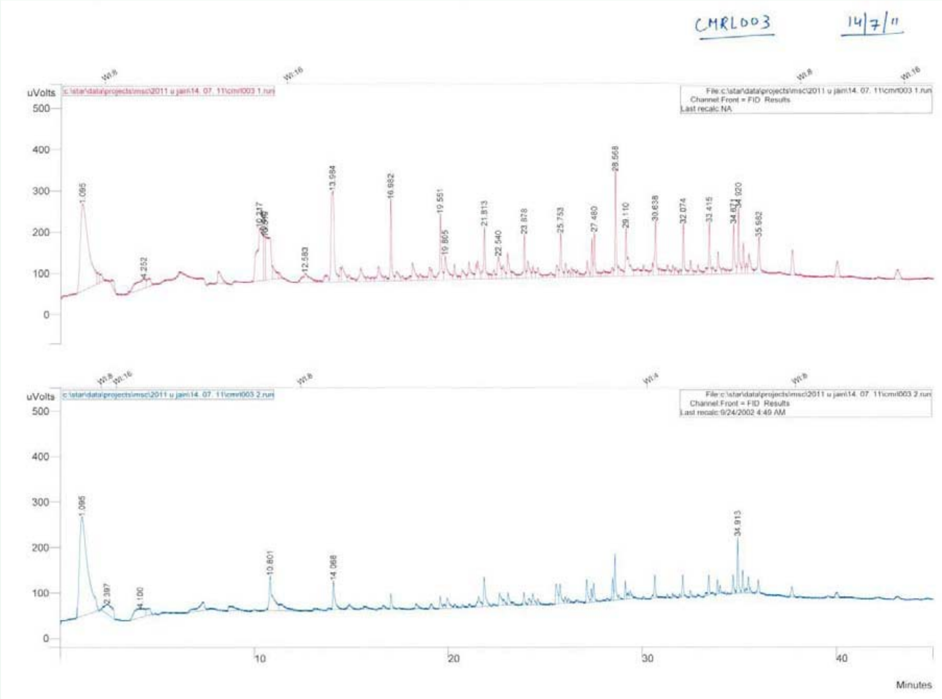

Figure 16 Peaks obtained in gas chromatograph for Breast milk sample CMRL003.

Print Date: Tue Sep 24 00:24:07 2002 Page 1 of 1

Title :
Run File : c:\star\data\projects\msc\2011 u jain\14. 07. 11\cmrl003 1.run
Method File : C:\star\Methods\Projects\MSc\2011 U Jain\Formula 2.mth
Sample ID : CMRL003 1

Injection Date: 9/24/2002 1:28 AM Calculation Date: 9/24/2002 2:13 AM

Operator : Richard
Workstation:
Instrument : GC CP3900
Channel : Front = FID

Detector Type: 39XL (1 Volt)
Bus Address : 44
Sample Rate : 20.00 Hz
Run Time : 44.992 min

** Star Chromatography Workstation Version 6.00 ** 02467-6490-c65-01e1 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		22.1691	1.095	0.000	6091	BV	27.4	
2		3.4123	4.252	0.000	938	BV	0.0	
3		9.8146	10.217	0.000	2697	BV	26.8	
4		2.1619	10.447	0.000	594	VV	26.8	
5		6.9120	10.519	0.000	1899	VV	49.5	
6		2.3639	12.583	0.000	650	BV	31.4	
7		9.1974	13.984	0.000	2527	VV	10.3	
8		3.6017	16.982	0.000	990	VV	4.0	
9		3.4095	19.551	0.000	937	VV	4.0	
10		2.2910	19.805	0.000	629	VV	8.3	
11		2.9027	21.813	0.000	798	VV	4.1	
12		2.0414	22.540	0.000	561	VV	8.5	
13		2.3300	23.878	0.000	640	VV	4.1	
14		2.1135	25.753	0.000	581	VV	4.4	
15		1.9990	27.480	0.000	549	VV	4.5	
16		3.9582	28.568	0.000	1088	VV	3.6	
17		3.8802	29.110	0.000	1066	VV	4.9	
18		2.9922	30.638	0.000	822	VV	4.9	
19		2.4055	32.074	0.000	661	VV	4.5	
20		2.2618	33.415	0.000	621	VV	4.6	
21		2.6624	34.671	0.000	732	VV	5.0	
22		2.7805	34.920	0.000	764	VV	4.0	
23		2.3393	35.982	0.000	643	VV	6.4	
	Totals:	100.0001		0.000	27478			

Total Unidentified Counts : 27477 counts

Detected Peaks: 100 Rejected Peaks: 77 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: 42 microVolts LSB: 1 microVolts

Noise (used): 2 microVolts - monitored before this run

Manual injection

****

Figure 17 Retention times obtained in gas chromatograph for Breast milk sample CMRL003.

(Figure A). Table (2) shows the volatile components found in the milk with their retention indices and characteristic flavor and aroma which could have been affected with the mother's diet/ milk ingredients.

In the Table (3) and graph (Figure A), all the eleven volatile compounds found to be present in the breast milk are listed with their peak areas. It was seen that the samples which showed the absence of a compound during the first run in the gas chromatograph was present in the second run which was carried out after some days of the first run. So, it showed that the composition of the milk with respect to its volatile component tends to change over a period of time on storage.

Similarly, Table (4) and (Figure B) shows the Peak areas of Volatile components found in formula milk sample. This outlines the effect of ingredients used (diet) on the volatile components in the formula milk

Comparing the major volatile compounds of formula milk with the breast milk, it can be seen that acetone is the only compound which is absent in the formula milk which has got a sweet and fruity flavor.

In Table (5) and (Figure C), the effects of diet on the volatile components in the semi skimmed milk are shown with the help of the peak areas of Volatile compounds found in semi-skimmed milk sample.

When the peak areas of semi-skimmed milk is compared with the breast milk it can be seen that Acetone, 2,4,7 tridecatrienal, and δ- decalactone are absent.

Two different comparisons about the amount of compounds present in different milk samples were made. First was between the three types of milk samples with similar constituents in order to find the difference in the presence of amount of volatile compounds present in the breast milk, formula and semi-skimmed milk, the samples were constant for formula milk and semi-skimmed milk, where as breast milk samples were different. Tables (6,7) + Figures (D,E) show the samples selected for the comparisons.

The sample of breast milk selected for the comparison (Table 6) has the same compounds as formula milk, where as the sample of breast milk (Table 7) contains all the compounds.

The comparison is clearly shown in the Figures (E and F).

Figure E: It can be seen that the amount of compounds present in the breast milk is much higher than the other milks, where as formula milk has more amount than semi-skimmed milk.

Figure F: The graph shows that formula milk's skyline dominating the rest.

The compound 7- hentriacontane shows more peaks and 20,24,28 tirmethyl tritriacontyl cyanide shows the least in comparing both the graphs in all types of milk.

Also, various diets that the mother consumed before providing the breast milk sample were compared to see its effect on the milk components. (Table 8) provides the list of corresponding food items/ diets consumed by the mother and volatile components ABSENT in the respective breast milk samples.

Analysis carried out by comparing the diet taken before getting different samples against volatile compounds found in each sample. Following interpretations could be made.

1. Based on the diet, it was separated based on few criterions to make the analysis easier like finding the influence of alcohol, smoking, tea, yoghurt, fruits, vegetables and meat.

2. Sample which showed the presence and the sample with absence of all volatile compounds were found out.

3. In order to find influence of alcohol and smoking, all tested

Table 3: All the eleven volatile compounds found to be present in the breast milk are listed with their peak areas in gas chromatograph].

S.no	Volatile compound found	BREASTMILK SAMPLES															
		CMR001	CML001	CMRL002-1	CMRL002-2	CMRL003	CMRL004	CMRL005	CMRL006	CM100A	CMR101	CML101	CM007	CM008	CM009	CM010	CM012
1	Acetone	1158	579	647		938	618				676	569	522			525	567
2	2,4,7- tridecatrienal	614		621			1048	686	1350							774	
3	Υ- nonalactone	1168	3302	1216	1926	2697	502	1482	816	589		1862	829	2292		1401	2799
4	δ- decalactone	869		698	1291	531	810		730	608		1519	750	1776	522	1001	653
5	4- hydroxy-3-methoxy benzaldehyde(vaniline)	555	811		657	798	1266	1020	2907		1645		599			652	1782
6	2-methyloctadecane					581	809	850	2760		985		1173				1311
7	7-hentriacontane	916	1536	819	630	1088	1858	2384	5960	675	2135	528	2843	896		976	2877
8	3,7,11,15 Tetramethyl hentriacontane		893	736		822	980	998	3000		1310		1419			672	1818
9	13-cis-cinnamoyloxy-17-oxolupanine		657	898		661	1008	1000	2678		1103		1284			683	1951
10	3,7,15 Trimethylpentatri-acontane	813	673	1093		764	1244	925	2123	807	920	502	1002	535		1257	1998
11	20,24,28 Trimethyl triac-ontane cynaide		665	952	563		620	657	878		530					589	

Table 4: Peak areas of Volatile components found in formula milk sample].

S.No.	Volatile Compound Found	Formula Milk Sample
1	Acetone	-
2	2,4,7- tridecantrienal	536
3	γ – nonalactone	1414
4	δ - decalactone	1502
5	4-hydroxy-3-methoxy benzaldehyde (vaniline)	2217
6	2- methyloctadecane	1657
7	7- hentriacontane	4456
8	3,7,11,15 Tetramethyl hentriacontane	2068
9	13-cis-cinnamoxyloxy-17- oxolupanine	1821
10	3,7,15 Trimethylpentatriacontane	1356
11	20,24,28 Trimethyl triacontane cynaide	850

Table 5: Peak areas of Volatile compounds found in semi-skimmed milk sample].

S.No.	Volatile Compound Found	Semi- Skimmed Milk Sample
1	Acetone	-
2	2,4,7- tridecantrienal	-
3	γ – nonalactone	2087
4	δ - decalactone	-
5	4-hydroxy-3-methoxy benzaldehyde (vaniline)	604
6	2- methyloctadecane	1052
7	7- hentriacontane	802
8	3,7,11,15 Tetramethyl hentriacontane	1963
9	13-cis-cinnamoxyloxy-17- oxolupanine	1205
10	3,7,15 Trimethylpentatriacontane	1438
11	20,24,28 Trimethyl triacontane cynaide	642

Table 6: Comparison of three types of milk (breast milk CMRL006)].

S.No.	Volatile Compound Found	Formula Milk	Breast Milk (CMRL006)	Semi-Skimmed Milk Sample
1	Acetone	-	1350	-
2	2,4,7- tridecantrienal	536	816	-
3	γ – nonalactone	1414	730	2087
4	δ - decalactone	1502	2907	-
5	4-hydroxy-3-methoxy benzaldehyde (vaniline)	2217	2760	604
6	2- methyloctadecane	1657	2760	1052
7	7- hentriacontane	4456	5960	802
8	3,7,11,15 Tetramethyl hentriacontane	2068	3000	1963
9	13-cis-cinnamoxyloxy-17-oxolupanine	1821	2678	1205
10	3,7,15 Trimethylpenta-triacontane	1356	2123	1438
11	20,24,28 Trimethyl triacontane cynaide	850	878	642

Table 7: - [Comparison of three types of milk (breast milk CMRL004].

S.No.	Volatile Compound Found	Formula Milk	Breast Milk (CMRL004)	Semi-Skimmed Milk Sample
1	Acetone	-	618	-
2	2,4,7- tridecantrienal	536	1048	-
3	γ – nonalactone	1414	502	2087
4	δ - decalactone	1502	810	-
5	4-hydroxy-3-methoxy benzaldehyde (vaniline)	2217	1266	604
6	2- methyloctadecane	1657	809	1052
7	7- hentriacontane	4456	1858	802
8	3,7,11,15 Tetramethyl hentriacontane	2068	960	1963
9	13-cis-cinnamoxyloxy-17-oxolupanine	1821	1008	1205
10	3,7,15 Trimethylpenta-triacontane	1356	1244	1438
11	20,24,28 Trimethyl triacontane cynaide	850	620	642

samples were compared to find volatile compounds that are commonly absent in the diet which included alcohol and cigarettes.

4. Similar comparison is made among samples in order to find influence of meat, fruits and vegetables included in diet.
5. A comparison is made for also finding out role of tea and desserts that were in diet.
6. Comparison between samples from different breasts after having same diet.

Sample CMRL004 is the only sample which showed presence of all volatile compounds.

The diet before sample was obtained is:

Chicken, ham, roast pork+ potatoes + carrots + leeks + Melon One Cigarette

Mint herbal tea

On contrary, sample CM009 is only where 10 out of 11 compounds were found to be missing.

The diet before the sample was taken is:

Mince + carrots + potatoes + leeks + 0% peach yoghurt

It can be seen that compounds 2,4,7- tridecatrienal, 3,7,11,15 Tetramethyl hentriacontane and 20,24,28 Trimethyl triacontane cyanide are commonly found missing in diets which included alcohol like wine, cava and ciders showing the influence of alcohol in the diet.

As evident from previous studies, lactating mothers who smoke, are at a greater risk of having a less successful lactation experience and are prone to suffer from 'insufficient milk syndrome' due to some hormonal imbalance and some harmful

Table 8: - [List of food items/ diets consumed by the mother and volatile components ABSENT in the respective breast milk samples].

Sample No.	Diet	Volatile Compounds not present
CMR001	2 glasses of cava, fish (salmon + cod) spinach + 0% peach yoghurt 3 cigarettes Herbal mint tea	2-methyloctadecane 7-hentriacontane 3,7,11,15 Tetramethyl hentriacontane 13-cis-cinnamoyloxy-17-oxolupanine 20,24,28 Trimethyl triacontane cyanide
CML001	2 glasses of cava, fish (salmon + cod) spinach + 0% peach yoghurt 3 cigarettes Herbal mint tea	2,4,7- tridecatrienal □ - decalactone 2-methyloctadecane
CMRL002-1	2 glasses of wine, chicken + roasted vegetables + rice noodles + Melon 4 cigarettes	4- hydroxy-3-methoxy benzaldehyd(vaniline) 2-methyloctadecane1`
CMRL002-2	2 glasses of wine, chicken + roasted vegetables + rice noodles + Melon 4 cigarettes	Acetone 2,4,7- tridecatrienal 2-methyloctadecane 7-hentriacontane 3,7,11,15 Tetramethyl hentriacontane 13-cis-cinnamoyloxy-17-oxolupanine 3,7,15 Trimethylpentatriacontane
CMRL003	2 glasses of wine, steak + potatoes + carrots + leeks + 0% vanilla yoghurt	2,4,7- tridecatrienal 20,24,28 Trimethyl triacontane cyanide
CMRL004	chicken, ham, roast pork+ potatoes + carrots + leeks + Melon 1 cigarette Mint herbal tea	
CMRL005	steak + couscous + courgettes + peppers + onions + tomatoes + aubergine 1 cigarette	Acetone □ - decalactone
CMRL006	lasagnes + 0% pink grapefruit yoghurt 1 cigarette Red bush tea	Acetone
CM100A	mince + courgettes + peppers + onions + tomatoes + pasta + apple 1 cigarette	Acetone 2,,7- tridecatrienal 4-hydroxy-3-methoxy benzaldehyde(vaniline) 2-methyloctadecane 3,7,11,15 Tetramethyl hentriacontane 13-cis-cinnamoyloxy-17-oxolupanine 20,24,28 Trimethyl triacontane cyanide
CMR101	1 magners, 1 glass of wine + steak + curry + cauliflower + rice + 0% lemon yoghurt 4 cigarettes Red bush tea	2,4,7- tridecatrienal ϒ- nonalactone □ - decalactone
CML101	1 magners, 1 glass of wine + steak + curry + cauliflower + rice + 0% lemon yoghurt 4 cigarettes+Red bush tea	2,4,7- tridecatrienal 4- hydroxy-3-methoxy benzaldehyde(vaniline) 2-methyloctadecane 3,7,11,15 Tetramethyl hentriacontane 13-cis-cinnamoyloxy-17-oxolupanine 20,24,28 Trimethyl triacontane cyanide
CM007	1 glass of wine + Moroccan chicken kebab + green beans + potatoes + carrots + plum and blackberry tart + 4 cigarettes + Red bush tea	2,4,7- tridecatrienal 20,24,28 Trimethyl triacontane cyanide
CM008 (HINDMILK)	1 glass of wine+ pizza + raw carrots + ham + apple + toast + cheese + 0 cigarette	Acetone 2,4,7- tridecatrienal 4- hydroxy-3-methoxy benzaldehyde(vaniline) 2-methyloctadecane 3,7,11,15 Tetramethyl hentriacontane 13-cis-cinnamoyloxy-17-oxolupanine 20,24,28 Trimethyl triacontane cyanide

CM009	mince + carrots + potatoes + leeks + 0% peach yoghurt + 0 cigarette	Acetone 2,4,7- tridecatrienal ⍰ - nonalactone 4- hydroxy-3-methoxy benzaldehyde(vaniline) 2-methyloctadecane 7-hentriacontane 3,7,11,15 Tetramethyl hentriacontane 13-cis-cinnamoyloxy-17-oxolupanine 3,7,15 Trimethylpentatriacontane 20,24,28 Trimethyl triacontane cyanide
CM010	roast pork + courgettes + aubergines + couscous + melon+ 0 cigarette	2-methyloctadecane
CM012	ham + cheese + cucumber +salad + tomatoes + bread + apple+ 0 cigarette	2,4,7- tridecatrienal 20,24,28 Trimethyl triacontane cyanide

elements like nicotine, cotinine, etc [13]. However, not much missing components were observed in the present study that were absent specifically because of cigarette (on comparison between diets with and without cigarettes).

Acetone was absent in samples that where diet contained rice, pizza and pasta which are basically cereal group and are rich source of carbohydrates.

Also, Vanillin was absent in the diet which doesn't include tea or yoghurt, while, many compounds were missing where diet contained mince and steak.

DISCUSSION

Hausner et al., [14] found 54 volatile compounds collectively from all samples taken from ten different women. In this experiment, 11 volatile compounds have been found from sixteen different samples but from the same person. Hausner's study helps in understanding that there are more volatile compounds in breast milk present than the ones identified in this project and also can be understood that compounds found in milk differs from one person to another based on various factors.

Based on analysis, influence of diet on presence/absence of volatile compounds has been made. It can be seen that presence/ absence of carbohydrates in diet highly influences presence of acetone in milk samples been tested.

The amount of carbohydrates present in rice, pizzas, pastas and other dishes that include wheat etc, is relatively high compared to other foods. It is one of the behavior of a body to produce acetone when body does not have any instant source of energy like carbohydrates, hence, producing ketone bodies during the process of fat metabolism to extract energy. Acetone is one of the components of three ketone bodies produced with a characteristic flavor and aroma. As there are chances for presence of more carbohydrates in sample, containing rice, and wheat in diet, acetone is generally absent.

Also, it can be explained that alcohol consumption and smoking highly influences presence of volatile compounds in the breast milk. It was seen that some important volatile compounds were found missing in diets with alcohol when compared to diets with same foods without alcohol has produced comparatively more compounds.

Vanillin is a sugar containing compound, it was noted from results that the compound is present in the samples where yoghurt is included in the diet. It can be due to the flavor of vanillin present in the yoghurt.

While considering meat, based on results, beef has a bad influence on the volatile compounds, as diets with minced meat and steak showed absence of many compounds compared to other meat forms. Better results were obtained with samples having more compounds that contained chicken and pork. The samples that contained pork showed much better results than those samples with chicken.

When sample CMRL004 (contains all the volatile compounds) was analyzed, it was observed that diet had a combination of chicken, meat, vegetables, fruit and desserts.

It was also inferred that samples collected on the same time but from the different breasts showed a considerable difference concerning its volatile components even with same diet.

For example: in the case of sample CMR001, CML001 and CMR101, CML101.

Here, CMR001 shows absence of five components whereas CML001 shows absence of only three components.

On the other hand, CMR101 has three components lacking whereas CML101 has six.

Therefore, showing that not only diet, but anatomy and physiology of different breasts also affect presence of volatile components in breast milk.

However, hind milk in sample CM008 does not show much of different volatile components profile when compared to other milk samples. It showed absence of seven compounds out of eleven compounds that can be well explained with the diet consumed. One glass of wine+ pizza + raw carrots + ham + apple + toast + cheese + zero cigarette was consumed which doesn't seem to a very good combination of foods to provide all components.

In addition, each compound present is varying in amounts in different sample which is shown with the help of the (Table 3) and graph (Figure A) of the peak areas. For example: acetone is present in both CMR001 and CML001 but their peak areas are vastly different, i.e. 1158 and 579 respectively, also Ɣ-nonalactone concentration in samples CMRL003 and CMRL004 is 2697 and 502 respectively, indicating differences in amounts of a same compound in two different samples.

When these breast milk samples were compared with formula milk and semi-skimmed milk samples, it was found that there were some compounds, which were missing in formula and semi-skimmed milk. In addition, components which were present, varied in quantities, which have been demonstrated using two graphs (Figures D,E) + (Tables 6,7) as an example where formula milk and semi-skimmed milk have been compared with different breast milk samples. Composition of these three types of milks was found to be a bit same with different quantities of components as some being high and some being low being dependent on diet and other factors in the breast milk and in the other milk, it was taken for one sample only and hence, constant, causing difference in flavor of the different milk samples.

CONCLUSION

From above experiment, it was concluded that there are few similarities and dissimilarities in the compositions of breast milk, formula milk and semi-skimmed milk. The eleven volatile components we came across on testing the milk samples have different flavor and aroma that play a role in changing the sensory profile of the respective milk.

However, cow's milk is used in the preparation of the formula milk in the factories; even then, there was difference in their compositions. Formula milk showed the absence of only one compound, i.e. acetone whereas semi-skimmed milk showed the absence of three compounds.

This can be because manufacturers can alter the composition of formula milk by adding/deleting some ingredients that is almost impossible with cow's milk. So, the composition of the formula milk is mostly constant.

Breast milk shows a more vast flavor and aroma profile as compared to formula milk and cow's milk. Hence, infants who are breast-fed are used to a diversity of tastes and odors affecting their food preferences and openness to different flavors later in life.

Future research: However, a future research can be done on testing how differently infants (fed on different kinds of milks) responds to various flavors and food choices upon weaning or a little later in life.

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