Identification of Functional Therapeutic Compounds in *Moringa oleifera* Fortified Bio-yoghurt

Enaam S. Shokry[1] , Susu M. Alasfoury[2], Eman M. Ibrahim[3]

ABSTRACT

Presently, the consumption of probiotic yogurt has been a significant increase. Mostly consumers prefer to take probiotic yoghurt because of its taste and high nutrients. However, in the manufacturing process of probiotic yogurt, many fortified materials can be used. This research objective was to determine the effects and the quality properties of the locally produced dietary supplement probiotic yogurt with therapeutic compounds, fortifying conventional with probiotics *Lactobacillus acidophilus,* and *Bifidobacterium bifidum* as an effective approach for producing and particularly enhancing functional profits and its responses on human health have been investigated in various tested treatments. Ten bio-stirred yoghurt samples were prepared with different ratios of *Moringa oleifera* powder to improve and proof the nutritional quality of yoghurt and different treatments types of Stirred bio-yoghurt formulated and fermented with the normal yoghurt starter culture at 40 °C for 3 hr. After fermentation, control and tested bio-yoghurt treatments used for GCMS/MS analysis for detection the bio-active metabolites in the fresh bio-product and after cold storage at 5 °C for 2 weeks to check their shelf life. Fortifying probiotic yogurt with higher levels of *Moringa oleifera* powder significantly enhances a symbiotic state, provide health benefits and considers a very important prebiotic factor to the probiotic adjunct strains. The addition of leaves and seeds of *Moringa oleifera* powder including berberine compound is significantly optimizing the final products, gastrointestinal disturbances and avoid cardiac problems. Nevertheless, future studies are needed to correlate probiotic strains and specific health aspects to these bio-active molecules effectors.

Keywords*: Moringa oleifera,* Prebiotic, Probiotic strains, Bio-active compounds, Dietary supplement stirred bio-yoghurt.

INTRODUCTION

Fermented milk products are safe for all different ages this is because the presence of lactic acid bacteria (LAB), such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, are used (Mustika *et al.*, 2019). The popular consuming dairy products in the community and widely developed by food experts is yoghurt. Thus, yogurt is a sustainable, sour, refreshing milk product, more easily digested in the human gastrointestinal tract and one of other health benefits are specific probiotic strains e.g. immunological effects and production of bio-active compounds (Darwish *et al.,* 2022). A healthy lifestyle causes an increase concerning for enable people to easily get a sufficient amount of this probiotic dairy products via their daily diet. On the other hand The bio-availability of the adjunct cultures biosynthetic pathway enzymes support developing bio-yogurt production, increase of flavors and involving nutritionally healthy active compounds that can inhibit and reduce the growth of undesirable spoilage microorganisms in the bio-product therefore, suggests that moringa can act as a natural preservative (Maharani *et al.,* 2020). When regarding the chemical characteristics of moringa leave/seeds powder studies by Karamy *et al.* (2024) conducted that Moringa leaves powder can be used as a fortification, add nutritional value, and also incorporating moringa into yogurt can extend the shelf life, enhance and improve products quality of food products. Several studies on the health value of *Moringa oleifera* Based commonly on its functional ingredients, antioxidant activity and potential health benefits in yogurt production due to its rich in fiber 12.5 g, calcium 2185 mg, iron 25.6 mg, phosphorus 225 mg, protein 29.4 g, carbohydrates 41.2 g, and fat 5.2 g per 100 g (Isnan and Nurhaedah, 2017). All parts of *Moringa oleifera* may help in solving many nutritional deficiencies and can be used as a variety medicine; that is because its minerals content, and vitamins B, like pyridoxine, folic acid, and nicotinic acid. Moreover, *M. oleifera* leaves have a low calorific value and can be used in the diet (Rockwool *et al.,* 2013). Moringa seeds extract exhibit potential antimicrobial effects, can act as antifungal activity, anti-inflammation of the liver and antibacterial action against pathogens *Escherichia coli* and *Staphylococcus aureus* (Thomas, 1992). Higher concentrations may negatively impact taste and overall consumers acceptance. Therefore, careful optimizing of moringa concentration is essential and critical to maintaining the product's appeal to consumers (Trigo *et al.,* 2023). Therefore, the aim of this research was to investigate the effect of fortifying organic yogurt with different

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[1]Dairy Science and Technology Department, Faculty of Agriculture,
Alexandria University, Egypt. enaamshokery@yahoo.com,
[2]Taif University, P.O. BOX11099, Taif 21944 Saudi Arabia,
Agricultural Research Center, Food Technology Research Institute (FTRI), Egypt.
[3]Faculty of Agriculture, Kafr-El-Sheikh University, Egypt.

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dosages of moringa and its health impact, and bio-active polar and nonpolar volatile compounds.

MATERIALS AND METHODS

1-Moringa leaves\seeds powder preparation:

Moringa oleifera (*M. oleifera*) was obtained from the Agriculture Research Center El Doki, Ministry of Agriculture, Egypt. The use of Moringa leaves powder is also obtained through several processes, by sorting, washing, drying, and milling. Previous studies by Shokery *et al.* (2017).

2- Yoghurt Culture (primary starter):

The starter was obtained from Chr. Hansen Laboratories, Copenhagen, Denmark.

3- Preparation of Probiotic Culture (Secondary starter):

To prepare the probiotic culture, a modified procedure outlined by Segers and Lebeer (2014) was followed. Lyophilized probiotic starter (ABT culture) contains *Lactobacillus acidophilus,* and *Bifidobacterium bifidum*, the stock culture of was stored at − 70 °C. When used, it was propagated in sterilized skim milk and incubated for 48 h at 37 °C, followed by, inoculated 1.5% (7.48 log CFU/ml) to prepared milk or milk formulations, fermentation and coagulation carried out for bio-yoghurt formation. The pH was monitored during fermentation, and coagulation occurred when the pH reached 4.6 at the end of fermentation.

4- Bio- fortified stirred Yogurt production:

Fresh buffalo whole milk (8.1% fat, 9.5% SNF) was collected from animal production research, faculty of agriculture, Kafr-El-Sheikh University, Egypt. The five treatments of buffalo milk (8.1% fat & 9.5% SNF), control and four treatments were heated and fortified with different levels of *M. oleifera* (0.5 & 1% powder leaves and 0.5 & 1% powder seeds) to 90 °C for 7 min. Inoculation with ABT 10^6-10^7 CFU/ml starter culture at 40 °C for milk fermentation. Treatments were incubated at 42 °C until pH 4.7 and then cooled and kept at 5 °C overnight. Coagulated bio-yogurt was stirred by a mechanical mixer, filled in plastic bottles, and stored at 5 °C for 2 weeks as described by Habib *et al.* (2019). The treatment specifications are shown in Table (1).

5- Samples Preparation for Volatile Organic Compounds Analysis (VOCs):

If necessary, dry the sample to remove water, as moisture can interfere with silylation. Dissolve the sample in an anhydrous solvent such as pyridine, for Derivatization Reaction add 50–200 µL of (Bis trimethylSilyl fluoro-Acetamide) BSA (depending on the sample and analyte concentration). Incubate the reaction mixture at 60–80 °C in water bath for 15–30 minutes to ensure complete derivatization. Extraction of polar and non-polar metabolites from freeze-dried powder. The following protocol describes: by Shepherda *et al.* (2007). The general method used for extraction of metabolites from samples. Internal standards for polar metabolites (100 lL aqueous ribitol, 2 mg ml^{-1}) and non-polar metabolites (100 lL methanolic methyl nonadecanoate, 0.2 mg ml^{-1}) were added to powdered lyophilized yoghurt samples (100 mg) in a glass culture tube (125·16 mm). Methanol (3 ml) was added and the mixture was shaken vigorously on a vortex shaker at 30 °C for 30 min. Water (0.75 ml) and chloroform (6 ml) were added sequentially, and after each addition the mixture was shaken at 30 °C for a further 30 min. Finally more water (1.5 ml) was added and the mixture was shaken by hand and then separated by centrifugation into upper (polar) and lower (non-polar) fractions, each of which was isolated by pipette. The separated fractions were either subjected immediately to further processing, derivatisation and analysis, or alternatively were stored at 20 °C pending further processing. Polar fractions could be stored directly whereas non-polar fractions were first evaporated to dryness under nitrogen and then re-dissolved in iso-hexane containing 50 ppm 2,6-di-t-butyl-4-methyl phenol (BHT). 2.7. Linearity experiments to assess the linearity of the response ratios for individual metabolites with increasing sample size. Volatile compounds of the bio-yogurt samples were analyzed and identified similarly using a Thermo Finnigan Tempus GC–(TOF)–MS system.

Table 1. Treatments specifications

Treatment symbol	Specifications
C1	**Plain Stirred Yogurt fresh (zero week) (control)**
C3	**Plain Stirred Yogurt after storage (2 weeks) (control)**
T1-1	**Bio-Stirred Yogurt fortified with 0.5 % *M. oleifera* leaves fresh (zero week)**
T1-3	**Bio-Stirred Yogurt fortified with o.5% *M. oleifera* leaves after storage (2 weeks)**
T2-1	**Bio-Stirred Yogurt fortified with 1% *M. oleifera* leaves fresh (zero week)**
T2-3	**Bio-Stirred Yogurt fortified with 1% *M. oleifera* leaves after storage (2 weeks)**
T3-1	**Bio-Stirred Yogurt fortified with 0.5 % *M. oleifera* seeds fresh (zero week)**
T3-3	**Bio-Stirred Yogurt fortified with o.5% *M. oleifera* seeds after storage (2 weeks)**
T4-1	**Bio-Stirred Yogurt fortified with 1% *M. oleifera* seeds fresh (zero week)**
T4-3	**Bio-Stirred Yogurt fortified with 1% *M. oleifera* seed after storage (2 weeks)**

Conditions used for sample injection and chromatographic separation were identical to those described for the TOF system, except that a split of 80:1 was used on the PTV injector. The GC–MS interface temperature was 250 °C, and the MS source temperature was 200 °C. Mass spectra were acquired over the mass range 35–900 a.m.u. at 6 scans s^{-1} under EI ionization conditions at 70 eV, after a solvent delay of 1.25 min. Data were acquired using the Xcalibur™ software package V. 1.4.

RESULTS AND DISCUSSION

1-Volatile compounds of bio-stirred fortified fresh yoghurt:

To investigate the bio-active compounds GC-MS analysis was performed in the Start of study after direct fermentation and during 2 weeks of storage at 4 °C. The results demonstrated that the volatile compounds separated by GC-Mass spectroscopy in the moringa fortified bio-yoghurt at zero time were classified as alcohols, Esters, Aldehydes, Sterols, Ketones, and other compounds (Table 2; Figure 1 and stacked bar in Figure 2). Identified Alcohols and esters give the aroma and the flavoring properties of the probiotic product, this is in agreement with Amer *et al.* (2023). The volatile compounds can indicate accessibility that Lanosterol, Cholesterol and Inositol were the main found sterols in the bio-yoghurt samples, whoever, Bloj and Zilversmit (1997) stated that non-specific lipid transport protein" The sterol carrier protein-2' is believed to play an important role in lipid metabolism and CoA dehydrogenase synesis.

Thiophenecarbox aldehyde was detected in all treatments and control main while, -octadecanone Cyclohexanone, 2-Propanone,-Pentanone, 2 and 1-Butanone, ketonic compounds were isolated from all yoghurt treatment except (T 3-1) bio-yoghurt fortified with 0.5% moringa leaves powder. According to Ayad *et al.* (2004) results, ketones were the mainly defined volatile compounds. Among all treatments the common active compounds were Heptane, Bi cyclo heptane, Pregnan, and Hydrogen bromide Furthermore, Oxytetracycline antibiotic, lycopene, Digitoxin, and Mefloquine determined only with moringa seeds powder fortified treatment. The same results were regarded by Abdull Razis *et al.* (2014). Generally, as shown in stacked bar Figure (2) the recommended fortification ratio was moringa powder 1%, this is because the more functional bio-active compounds measured.

Table 2. GC-MS Profiling of volatile compounds in fresh bio-stirred fortified Yogurt treatments at zero time

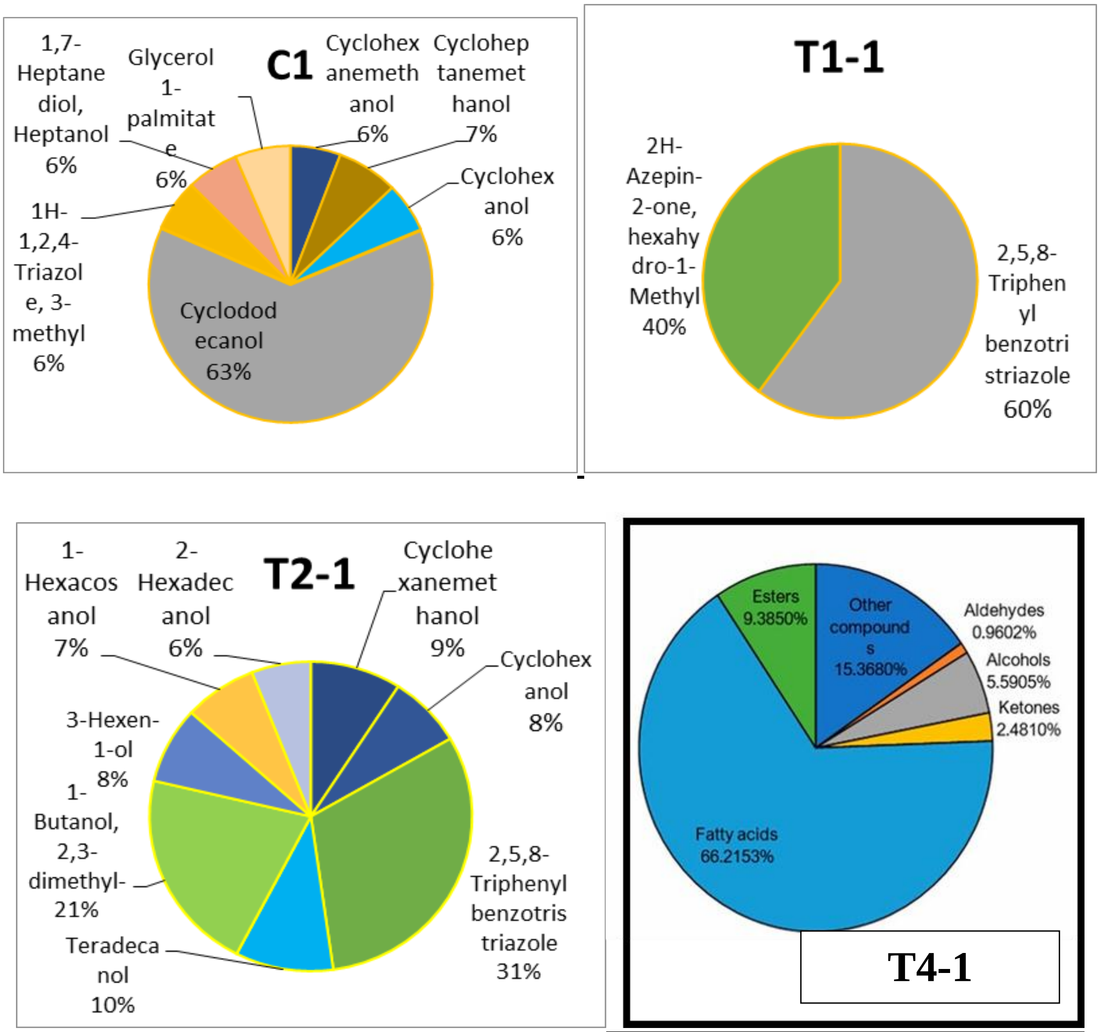
No	Compounds	Relative peak area %					Molecular weight (Mw) Daltons	Bio-function	Source
	a-Alcohols	**C1**	**T1**	**T2**	**T3**	**T4**			
1	Cyclohexanemethanol	(B) 0.67	(A)	(C) 1.64	(D) 0.90	(A)	154-156		
2	Cycloheptanemethanol	0.82		1.35		2.51	116-128		
3	Cyclohexanol	0.68					100		
4	Cyclododecanol				0.90	1.21	154-532		
5	2,5,8-Triphenyl benzotristriazol	7.38	1.22	5.60			74- 214		
6	Teradecanol			1.77			158	Yoghurt Aroma and Flavor Production	Krastanov *et al.* (2023)
7	1-Butanol, 2,3-dimethyl-		0.81	3.79	1.13		112-118		
8	2H-Azepin-2-one, hexahydro-1-Methyl				1.13		242		
9	1H-1,2,4-Triazole, 3-methyl	0.70		1.44			98		
10	3-Hexen-1-ol				0.56		382		
11	1-Hexacosanol			1.32			144		
12	-Methano-1H					2.77	382		
13	1,7-Heptanediol, Heptanol	0.70					270		
14	Phenol, 4-amino-2,6-dibromol	0.17					326		
15	Isopropyl 2,4-hexadienoate			1.07			158		
16	Glycerol 1-palmitate	0.74					60		
17	2-Hexadecanol					2.37	116		
18	Pr1-, opanol					1.53	242		
19	Octanol					2.54	242		
20	l-Decanol,					1.54	158		
	b- Aldehyde								
1	Thiophenecarboxaldehyde	0.70	1.22		1.22		59 -112	improve yoghurt sensory attributes and the quality, nutritional value, flavor, palatability, and shelf life	Mirza Alizadeh *et al.* (2025)

No	Compounds	Relative peak area %					Molecular weight (Mw) Daltons	Bio-function	Source
	c-Esters	**C1**	**T1-1**	**T2-1**	**T3-1**	**T4-1**			
1	Oxo hexadexyl ester	(B) 0.82	(A) 0.66	(C) 1.21	(D)	(A)	410-860		
2	Non anedioic dibutyl ester	0.86	2.55		1.71	1.71	300-860		
3	Methyl tridecyl ester	0.77	0.57				340-860		
4	Octanoic propenyl ester	0.77		2.25	1.26		184-624		
5	2,3 dihydroxy propyl ester	2.13					274		
6	9- octa decenyl ester	0.98		1.25	0.40	1.74	532-760		
7	2-Hydroxymethyl ester	0.83					214		
8	Oxo octadecyl oxy propyl ester	0.78		2.55		1.56	532-860	Low volatility and low Molecular weight Esters are bio-flavors responsible for Sensorial sensation	SÁ *et al.* (2017)
9	Butryic2- ethyl hexyl ester	0.75	0.11	1.40			100-378		
10	4-Decenoic ethyl ester	0.82					98	Aromatic esters confer Organoleptic impacts and creamy aromas	
11	Phosphoric acid tri octyl ester	1.10				11.35	834-860		
12	Imidazol ethyl ester				0.30	1.28	142		
13	Ethyl ester		0.57			1.39	834		
14	Lycopene ester	2.62	0.21	2.29	0.75	1.60	155-860		
15	Methyl ester	1.22	0.70		0.30		104-140		
16	Acetic acid, decyl ester			1.22	2.67	1.90	200-473		
17	Propanoic acid, hexyl ester				0.39		158		
18	Ethylene glycol diallyl ether				0.28		142		
19	nitro-, acetate (ester				0.39		150		

					d-Sterols				
1	Lanosterol	0.86					426	Sterols as a crucial lipid molecules presents in all eukaryotic cells ,1- maintaining membrane fluidity , 2-acting as signaling molecules for signal transduction pathways , 3- important precursors for fat soluble Vitamins and several Hormones .	García *et al.* (2000)
2	Cholesterol	8.62					368		
3	Inositol		0.31	1.19			340-764		
					e- Ketones				
1	3-octadecanone	0.75			0.32	1.55	268	concentration of, ketones Enhance The odour, flavour and taste sensory in yoghurt, derived from milk off-flavours led to the reduction of Ketones formation	Sfakianakis and Tzia (2017)
2	Cyclohexanone	7.55			0.45	2.90	143-154		
3	2-Propanone			3.28			194		
4	-Pentanone, 2			0.99			100		
5	1-Butanone, 1				0.45		154		
					f-Other Compounds				
1	Heptane	1.31	1.32		0.17		198	Used to block gap junction increase axial resistance between myocytes for increase heart's susceptibility to reentrant excitation and sustained arrhythmias.	Syed *et al.* (2024)
2	Bi cyclo heptane	1.22					96		
3	Strophanthidin					2.56	414	heart failure diseases	Ali *et al.* (2013)
4	Permethrin	1.00				3.80	404-536	Antioxidant bio-accumulative enzyme bioindicator role in food webs	Günal *et al.* (2021)
5	Ascorbyl palmitate	0.98				2.16	390-654	Good antifungals	Ali *et al.* (2013)
6	Carotene	4.50					414	Antioxidant effects, cell gap junction-related functions and immune-related functions and protective effect on gastric cancer	Chen *et al.* (2021)
7	Berberine		0.60	1.30		1.81	536-589	an antimicrobial in the treatment of dysentery and infectious diarrhea. multiple cellular mechanisms., arrhythmias and/or heart failure.	Lau *et al.* (2001)
8	Hydrogen bromide		0.36	1.45			125-654	generated bromination method at room temperature.antibacterial activity against gram-negative *E. coli* and *B. cereus*	Shivangi *et al.* (2024)

9	Xanthylium,	0.39	2.11			80-536	Pigment used as coloring agent , **Antioxidant activity** xanthophyll cycle.	Jully *et al.* (2016)
10	Pyridine			0.43		536	important natural products, including niconine, nicotine, and nucleic acid high therapeutic properties chemotherapeutic agents.	Prasad *et al.* (2018)
11	Lycopen	0.75		0.43		79	protect from damage caused by UV and photoaging	Mashuri *et al.* (2019)
12	Oleic acid		2.05	0.50	2.51	282	Antinflmtatory induced substant	Santa-María__*et al.* (2023)
13	2-Propenoic acid,		2.29			184	Pro anti-inflammatory, antibacterial and anthelmintic activity eliminate the spreading of bacteria or viruses and start the process of healing	Konopelski and Mogilnicka (2022)
14	Caprylic anhydride				1.43	270	antimicrobial properties and Minimum bactericidal concentrations	Valipe *et al.* (2011)
15	DL-Arabinose		0.98			150	low-calorie foods . chiral diastereomers that can exhibit specific co-crystallization agents in pharmaceutical formulations	Tyson *et al.* (2022)
16	L-(+)-Rhamnose			0.34		127	antibacterial agents, valuable for tumor immunotherapy.	Li *et al.* (2022)
17	Quinuclidine-3-ol				1.76	164	muscle contraction, cognition, and autonomic nervous system regulation.	Žunec *et al.* (2024)
18	Decanoic acid, silver(1+) salt				1.77	278	edible food additive for improving taste	Chowdhury *et al.* (2021)
19	Oxytetracycline				1.40	460	used for infections caused by a wide spectrum of bacteria infections respiratory tract (pneumonia), urinary tract, soft tissues, and skin	Li *et al.* (2020)
20	Cytosine				1.55	557	Active enzymatic deamination of cytosine beneficial and detrimental implications on various cellular processes n organismal evolution.	Shapiro (1999)
21	Mefloquine				1.54	111	antimalarial	Ali *et al.* (2013)

a-Alcohols



b-Aldehydes

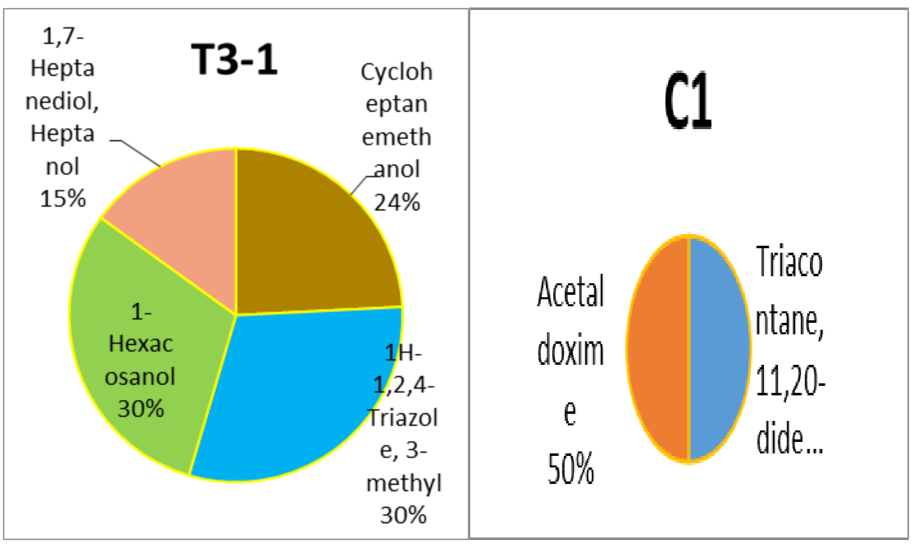


Fig. 1. Pie Charts of Volatile compounds by GC-MS analysis of bio-stirred fortified yoghurt treatments at zero time

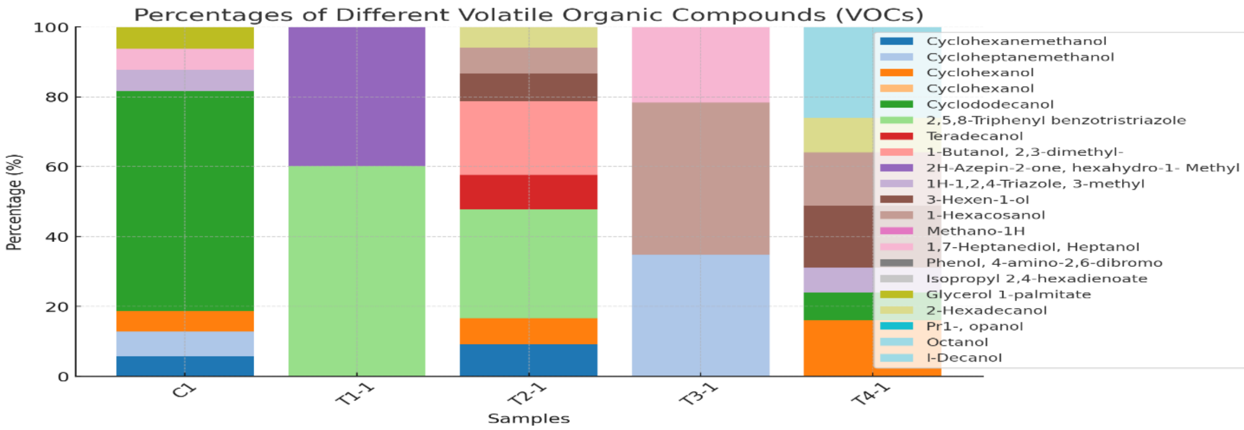


Fig. 2. The percentages of volatile organic compounds (VOCs) in the different samples (C1, T1-1, T2-1, T3-1, T4-1) using stacked bars

2-Volatile compounds of bio-stirred fortified yoghurt in the end of study after storing at 4 °C for 2 weeks

Regarding data in the Table (3) and Figure (3), there was a strong positive correlation between the moringa percent in the functional bio-stirred yogurt and the volatile bioactive detected profiles. concluded that the obtained results revealed that the approximate percent of identified volatile compounds was distributed as follows: esters 50%, alcohol 15%, other compounds 20%, ketones 8%, aldehydes 5%, and sterols at the lowest percent 2%. The same finding was recorded with Ayad *et al.* (2004). Polar volatile compounds, alcohols, and esters are the highly determined percent and responsible for yogurt aroma flavor, while sterols are formed after two weeks of cold storage. Also, Ali *et al.* (2013) mentioned that the concentration of metabolites depends on the storage temperature and storage period. Control treatments included plain bio-yogurt at fresh and 2-week periods (C1, C3) with adjunct cultures (*Lactobacillus acidophilus, Bifidobacterium bifidum*) without moringa fortification, enhancing functional promising metabolites, which exhibit nutraceutical functions, i.e., strophanthidin, which is used in heart failure diseases, ascorbyl palmitate, and pyrones, which are considered good antifungals, and xanthylium pigment in the C1 treatment. Mefloquine is an antimalarial, atorvastatin (reductase inhibitor, slowing cholesterol production), piperidine 4-methyl (anticancer and anti-inflammatory), acetyldigitoxin (phytosteroid used in cardiopathies), and sarcosine (inhibitor to glycine receptor) in C3 treatment. In comparison, the probiotic bacteria showed almost a similar pattern, where the *Bifidobacterium spp*. has a biological role in human health that can be maintained at the initial levels and then decreased slightly by 32 weeks (Ali *et al.*, 2013). Fortification with *M. oliefera* leaves powder at levels 0.5% and 1% in treatments T1-1 and T1-3, respectively, plays an important role as a prebiotic. The main effective metabolites profiling produced are heptane, hydrogen bromide, pyridine, oleic acid and cabrylic anhydride with T1-1 while, tripalmitin, lycobene, quinquepheryl, hexasiloxane and zeaxanthin with T 1-3 treatment. Several reports have indicated that there are 27 major compounds in leaf extracts. Most of these compounds pose different biological roles and give symbiotic and well-being welfare to the consumers (El-Ziney *et al.*, 2017 and Shokery *et al.*, 2017).

Stacked bar in Figure (4) illustrates the identified phytochemical compounds by GC-MS in the water-ethanol extract of the *M. oliefera* seeds powder fortified in the bio-yogurt (T4-1 and T4-3) samples. The most abundant compounds were Mefloquine, Bupropion (major depressive and stop smoking programs). Bemegride (respiratory stimulator), Pyrimidi netrione (Boron) (bone health and affect estrogen/testosterone), Rescinnamine, Glaucine (decrease sleepiness), and Decanoic acid, silver (1+) salt (edible food additive for improving taste). These compounds improve food taste, and they have an impact on health as antioxidants, anti-inflammatories, anti-atherosclerosis and antimicrobial agents. The obtained results by Amer *et al.* (2023) using GC-MS recorded that thirty-eight volatile compounds were identified. Several epidemiology studies have suggested the adequate intake of lycopene at skin level it seems to protect from damage caused by UV and photoaging (Mashuri *et al.*, 2019).

Table 3. GC-MS analysis of volatile compounds in bio-stirred fortified Yogurt treatments after (2weeks)

No	Compounds	Relative peak area %					Molecular weight (Mw) Daltons	Bio-function	Source
	a-Alcohols	**C3**	**T1-3**	**T2-3**	**T3-3**	**T4-3**			
		(B)	(A)	(C)	(D)	(A)			
1	Hexacosanol	1.14					382		
2	Glycerol 1-palmitate	1.23			1.15		176-330		
3	2-Hexadecanol	1.48					242		
4	Phenol, 4-amino-2,6-dibromo	2.64				1.01	112-265		
5	Cyclohexanemethano	0.97					114		
6	Cyclohexanol	2.47	0.11				116-132		
7	1,7-Heptanediol	2.47	0.31	1.00			132-156		
8	1-Butanol, 2,3-dimethyl-	2.22		3.25			242		
9	1-Decanol, 2-hexyl	0.97					88		
10	1-Propanol, 2,2-dimethyl-	1.82	1.00	1.53	2.12	0.38	144-242		
11	5-Amino-2-methoxypheno l		2.97			0.37	60-88		
12	3-Pentanol	1.70	0.14		2.91		88-702		
13	-Indenol,	0.70	0.56	1.51			59-98		
14	Cholest-5-en-3-ol,	1.16	0.06			4.35	150-448	Yoghurt Aroma and Flavor Production	Krastanov *et al.* (2023)
15	Behenic alcohol	1.27					310		
16	1-Tricosanol	0.97		2.49			378-386		
17	2-Furanmethanol		0.34				\473		
18	Phytol			1.59			340		
19	Silanol, trimethyl				2.91		314		
20	1-Octanol, 2-butyl-		0.17	1.51	2.12		98-326		
21	Ethanol, 2-(dodecyloxy		0.21		0.97		230-683		
22	1-Tricosanol			1.30	1.05		340-444		
23	Isopropyl Alcohol				2.01		60		
24	1-Undecanol				1.25		186		
25	2-Dodecanol					1.25	172		
26	Lanostan-3á-ol,					1.30	444		
27	3-Tetradecanol					1.34	214		
28	2-Hexadecanol					0.58	242		

No	Compounds						Molecular weight (Mw) Daltons	Bio-function	Source
	b- Aldehyde								
1	Triacontane, 11,20-didecyl		1.20				506	improve yoghurt sensory attributes and the quality, nutritional value, flavor, palatability, and shelf life	Mirza Alizadeh *et al.* (2025)
2	Acetaldoxime		1.20				506		
No	**Compounds**	**Relative peak area %**					**Molecular weight (Mw) Daltons**	**Bio-function**	**Source**
	c-Esters	**C3**	**T1-3**	**T2-3**	**T3-3**	**T4-3**			
1	methyl ester	1.20		1.72	1.32	0.45	171-654		
2	dimethyl ester		0.26				624		
3	ethenyl ester	.			1,27		834		
4	diethyl ester	1.31					128		
5	Octadecanoic acid, ethenyl ester	3.78				2.87	310-330		
6	tetradecyl ester		0.16				860		
7	hexadecyl ester	0.08				.	370		
8	2-Propenoic acid, ESester	3.40	0.64		1.20		296-472	Low volatility and low Molecular weight Esters are bio-flavors responsible for Sensorial sensation Aromatic esters confer Organoleptic impacts and creamy aromas	SÁ *et al.* (2017)
9	methylpropyl ester	0.93					440		
10	Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	2.36	0.17	2.62			200-390		
11	2,3-dihydroxypropyl ester	2.90				3.49	390		
12	4-nitrophenyl n-Caprylic acid isobutyl ester	1.82		2.03	1.67		165-472		
13	1,2-enzenedicarboxylic acid, diisooctyl ester			2.10			233		
14	Carbamic acid, methyl-, 3-methylphenyl ester	2.27		1.40			428-472		
15	Benzoic acid ester	2.27		1.83			260-428		

16	propyl ester	3.77		1.35			426-434		
17	-Alanine,ester		0.17				860		
18	2-Butenedioic ester		1.65				268		
19	Decanoic acid, 2-propenyl ester			0.26			624		
20	2,3-dihydroxypropyl ester	1.76					234		
21	Phosphoric acid, trioctyl ester					0.83	412		
22	1,2,3-propanetriyl ester				1.36		472		
23	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester				1.76		370		
24	2-Butenoic acid, ethyl ester					0.68	114		
25	Ethanimidic acid, ethyl ester					0.37	87		
							d-Sterols		
1	Stigmasterol	3.73					212	Sterols as a crucial lipid molecules presents in all eukaryotic cells ,1-maintaining membrane fluidity , 2-acting as signaling molecules for signal transduction pathways , 3-important precursors for fat soluble Vitamins and several Hormones .	García *et al.* (2000)
2	Lanost-8-en-3-ol	1.61					638		
3	Lanosterol	1.04					412		
							e- Ketones		
1	Cyclohexanone	1.30			2.21	1.18	140-154		
2	Cyclohexanone, 4-dimethylpropyl)	1.65					168		
3	2(3H)-Furanone	1.48	0.25				268-282		
4	3-Octadecanone			1.72			268		
5	Ethanone, 1-	0.93					268		
6	cyclohexyl-Bentazon	2.90					126		
7	Hexahydro-2(1H)-azocino ne	1.03					240		

8	11-Heneicosanone	0.94				7.29	127-310		
9	2-Propanone	1.27					310		
10	Pantolactone	0.12		8.12			310		
11	-Heneicosanone			8.12			310	concentration of, ketones Enhance The odour, flavour and taste sensory in yoghurt, derived from milk off-flavours led to the reduction of Ketones formation	Sfakianakis and Tzia (2017)
12	3-Octadecanone			1.72			130-194		
13	2-Butanone, 3-				1.17		86		
14	methyl- 3-Heptanone				2.02	1.04	114-142		
15	3-Octadecanone				3.43		268		
16	-Heneicosanone				2.00		310		
17	Benzofuranone,				1.03	1.36	112-282		
18	1,4-Cyclohexanedione					2.91	184		
					f-Other Compounds				
1	**Mefloquine**	**1.16**			**0.39**	**0.42**	**378**	**antimalarial**	**Ali *et al.* (2013)**
2	Antimycin A2	3.78					534	species of Streptomyces, Antimycin A is a mixture of A1a and A1b isomers;An antifungal toxin (inhibits fungal food	Liu *et al.* (2023)
3	Atorvastatin	1.61					558	reductase inhibitor, slowing cholesterol production)	Amer *et al.* (2023)
4	Oleic Acid	1.76					282	Anti-Inflammatory_induced substant	Santa-María *et al.* (2023)
5	Piperidine, 4-methyl	1.19	0.29				99-282	(anticancer and anti-inflammatory)	Srividya *et al.* (2023)
6	Acetyldigitoxin	0.94			.		806	phytosteroid used in cardiopathies	Amer *et al.* (2023)
7	Bromophenol blue	1.27					666	lysozyme–bromophenol blue binding system	Akpomie *et al.* (2024)
8	4,6-Dihydroxypyrimidine	1.64					112	Broad-spectrum antimicrobial agents are often useful for the management of phytopathogenic fungi, bacteria, and cyanobacteria	Zhou *et al.* (2024)

								species.	
9	Sarcosine	3.09	0.21	2.10			233-654	inhibitor to glycine receptor , a Potential Prostate Cancer Biomarker	Cernei *et al.* (2013)
10	Fenoxanil	3.09	0.16			2.83	59-233	mycotoxins pesticide residues	Yan *et al.* (2024)
11	Guanidine		78.94				104	Guanidinium-based transporter \vectors acting as Antithrombotic ,Antidiabetic, Chemotherapeutic agents ,Anti-Inflammatory ,Na+\H+ Inhibitor Exchanger	Gomes *et al.* (2023)
12	Tetrafluorohydrazine		0.21		1.17	0.31	86-233	Used in organic synthesis and as an oxidizing agent	Daddiouaissa *et al.* (2021)
13	Berberine		0.39				256	an antimicrobial in the treatment of dysentery and infectious diarrhea. multiple cellular mechanisms., arrhythmias and/or heart failure.	Lau *et al.* (2001)
14	n-Hexadecanoic acid		0.20	1.13		0.80	654-806	antioxidant and antibacterial activity	Ganesan *et al.* (2024)
15	Tripalmitin		0.91				458	inhibits glucose-stimulated insulin secretion and reduces viability of INS1 cells in a dose-dependent manner. Formulations containing Tripalmitin are used in cosmetic products to condition skin and as a thickening agent. Tripalmitin is also used to form the lipid matrices of nanoparticles for drug delivery.	Pellegrino *et al.* (2022)

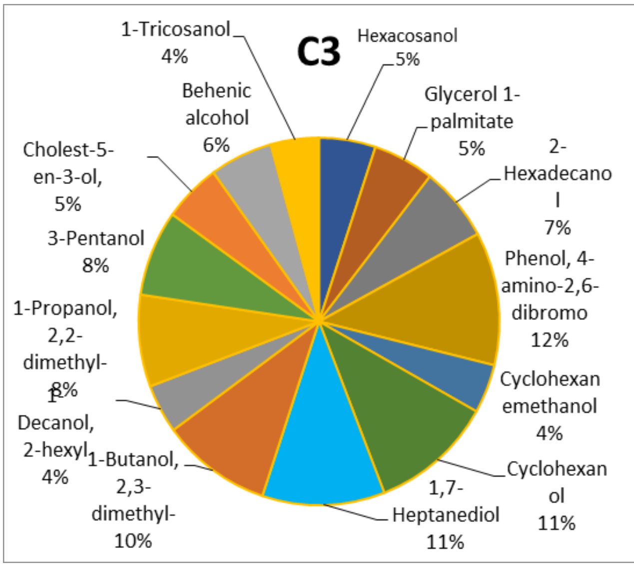
16	Hexasiloxane,	0.24				382	carbohydrate 3-O-Methyl-d-glucose has been reported to antimicrobial effects , anti-inflammatory and antioxidant effects	Youssef *et al.* (2023)
17	Quin quephenyl	0.34				536	anticancer, antihypertensive, antimicrobial, antifungal, antibacterial, analgesic, anti-inflammatory, antituberculosis, and antimalarial activity	Al-Kaf and Al-Robaidi (2024)
18	Lycopene	0.29				338	protect from damage caused by UV and photoaging	Mashuri *et al.* (2019)
19	Erucic acid		2.76	0.93	0.80	536-624	antioxidant and anti-inflammatory effects and it is a ligand of PPAR-δ. EA may act beneficial in Multiple Sclerosis and Alzheimer's Disease.	Altinoz and Ozpinar (2019)
20	Zeaxanthin	0.41			0.42	378-568	Pigment used as coloring agent , protective of macular in the eye, and in maternal and infant health.	Li *et al.* (2023)
21	Piperidine, 1-acetyl			0.88		568	pharmacology. Employing DFT techniques,	Abdelshaheed *et al.* (2021)
22	Demeclocycline		1.14			127	exhibit anti-viral, anti-infammatory, and immunomodulatory activities, mild-to-moderate COVID-19	Iwahori *et al.* (2023)
23	Glycine		1.20	1.32		171-464	Pro anti-inflammatory lowering free fatty acids improve the insulin response,	Aguayo-Cerón *et al.* (2023)

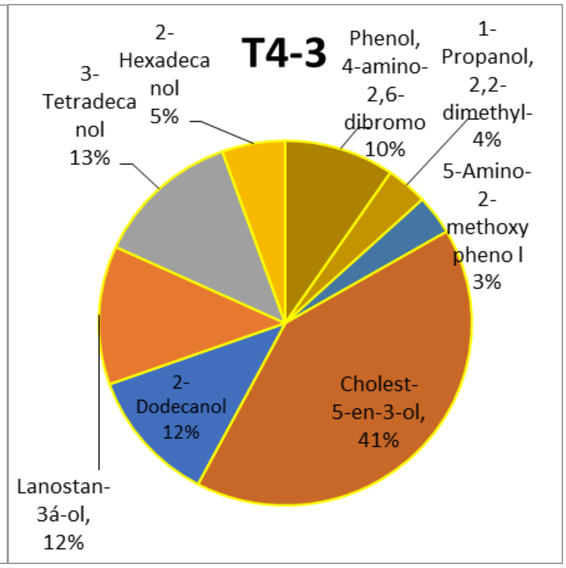
24	Ascorbyl Palmitate	1.45	1.27	7.29	75-414	food industry antioxidant, preservative, especially for preventing rancidity in fats\ oils. cosmetics and pharmaceuticals	Imran *et al.* (2024)
25	D-Glucose,	1.13			414	The obligatory cellular energy source , signaling molecule for glucose-sensing and proteins ,yield phosphorylation energy molecules	Dey *et al.* (2022)
26	á Carotene	2.76	0.93		536	Antioxidant effects, cell gap junction-related functions and immune-related functions and protective effect on gastric cancer	Chen *et al.* (2021)
27	Pyrimidi netrione(Boron)*		1.27	0.36	360-536	bone health and affect estrogen/testosterone)	Amer *et al.* (2023)
28	Normorphine		2.61	.	271	restores contractile function, ischemic tolerance, mitochondrial structure and function, and membrane dynamics	Zemljic-Harpf *et al.* (2021)
29	Cholic acid		2.61		271	Bile salt help in nutreients absorption and excretion of toxic metabolites .	Qian *et al.* (2022)
30	1H-Imidazole		1.50	0.26	93-98	fungicides, herbicides, plant growth regulators anticancer, antifungal antiviral , antibacterial antitubercular, antiparasitic, antihistaminic, anti-inflammatory, anti-neuropathic, anti-obesity, and antihypertensive effects, other therapeutic functions	Almarhoon *et al.* (2024)_

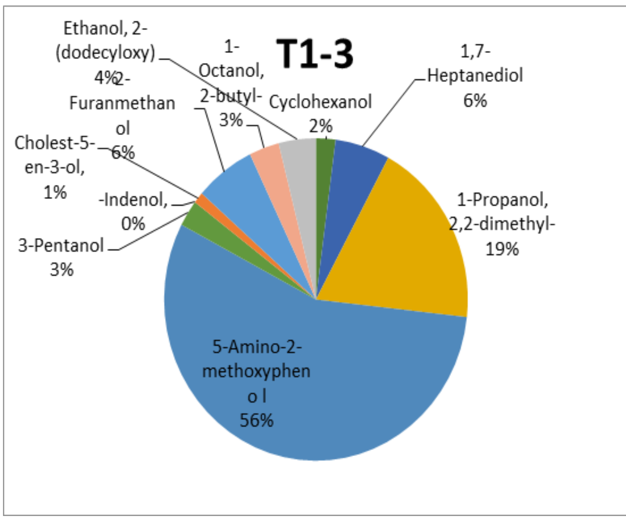
31	Chlortetracycline	1.54	478	enhances performance via improving digestive enzyme activities, antioxidant capacity, anti-inflammatory function	Long *et al.* (2021)
32	Rescinnamine	1.54	634	decrease sleepiness	Amer *et al.* (2023)
33	Bemegride	2.58	155	Respiratory stimulator, Activation Agent	
34	Quinoxaline	1.03	282	greener catalytic systemsantibacterial, antifungal, anticancer, anti-inflammatory, antiviral, and antiprotozoal activity	Sharma *et al.* (2022)
35	L-Tyrosine	1.76	523	an essential component for the production of several important brain chemicals called neurotransmitters, including epinephrine, norepinephrine, and dopamine. Neurotransmitters help nerve cells communicate and influence mood. Tyrosine also helps produce melanin, the pigment responsible for hair and skin color.	Boča *et al.* (2023)
36	l-Alanine	2.83	233	involved in sugar and acid metabolism, increases immunity, and provides energy for muscle tissue, brain, and the central nervous system.	Osborn *et al.* (2025)
37	D-Proline	2.07	115	used in peptide design to create a kink that promotes intramolecular folding and the formation of hydrogen bonds, leading to a β-hairpin conformation.	Azevedo (2019)

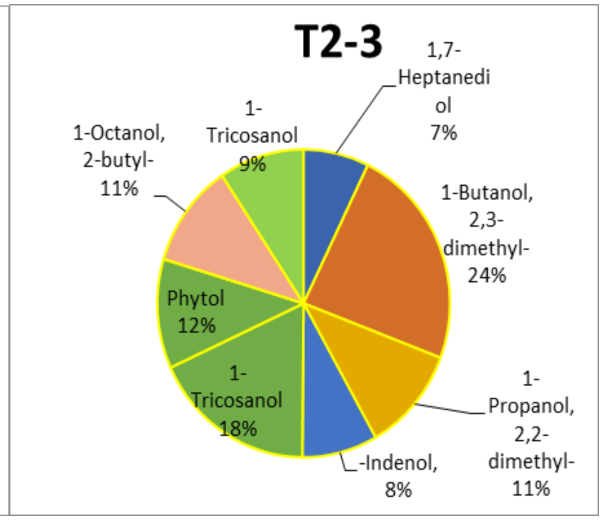
38	Glaucine	0.67	355	decrease sleepiness	Amer *et al.* (2023)
39	Bupropion	0.67	239	major depressive and stop smoking programs	Amer *et al.* (2023)
40	Propanoic acid	0.31	74	Pro anti-inflammatory, antibacterial and anthelmintic activity eliminate the spreading of bacteria or viruses and start the process of healing	Konopelski and Mogilnicka (2022)

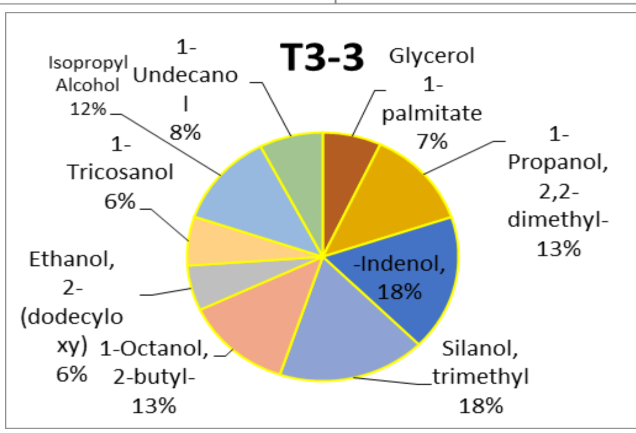
Alcohols


C3
Hexacosanol 5%
Glycerol 1-palmitate 5%
2-Hexadecanol 7%
Phenol, 4-amino-2,6-dibromo 12%
Cyclohexanemethanol 4%
Cyclohexanol 11%
1,7-Heptanediol 11%
1-Butanol, 2,3-dimethyl- 10%
1-Decanol, 2-hexyl 4%
1-Propanol, 2,2-dimethyl- 8%
3-Pentanol 8%
Cholest-5-en-3-ol, 5%
Behenic alcohol 6%
1-Tricosanol 4%

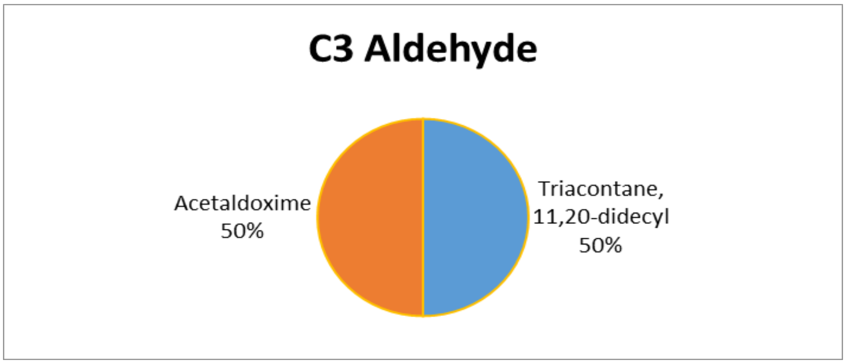

T4-3
2-Hexadecanol 5%
Phenol, 4-amino-2,6-dibromo 10%
1-Propanol, 2,2-dimethyl- 4%
5-Amino-2-methoxyphenol 3%
Cholest-5-en-3-ol, 41%
2-Dodecanol 12%
Lanostan-3á-ol, 12%
3-Tetradecanol 13%


T1-3
Ethanol, 2-(dodecyloxy) 4%
1-Octanol, 2-butyl- 3%
Cyclohexanol 2%
1,7-Heptanediol 6%
1-Propanol, 2,2-dimethyl- 19%
5-Amino-2-methoxyphenol 56%
3-Pentanol 3%
-Indenol, 0%
Cholest-5-en-3-ol, 1%
2-Furanmethanol 6%

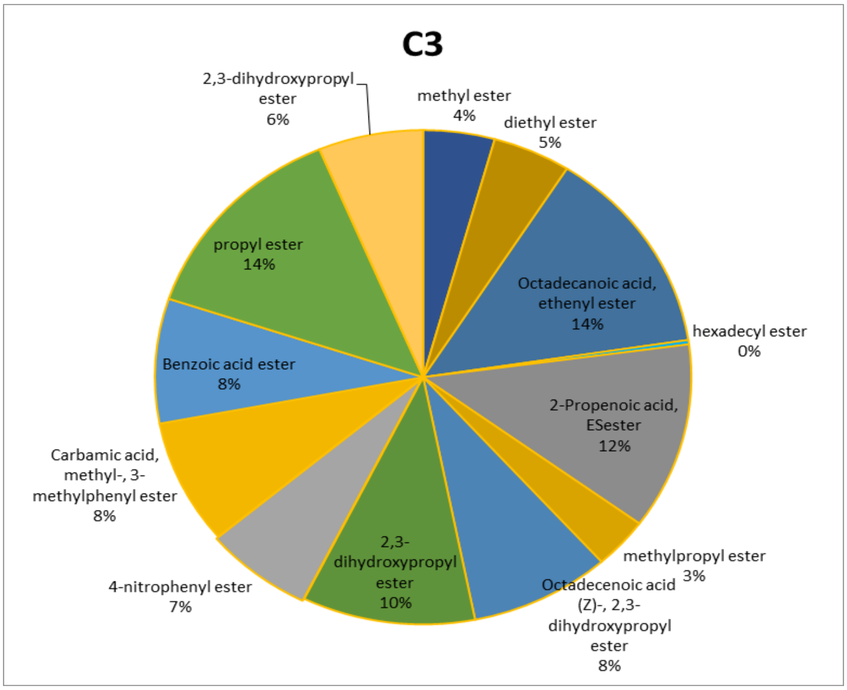

T2-3
1,7-Heptanediol 7%
1-Butanol, 2,3-dimethyl- 24%
1-Propanol, 2,2-dimethyl- 11%
-Indenol, 8%
1-Tricosanol 18%
Phytol 12%
1-Octanol, 2-butyl- 11%
1-Tricosanol 9%

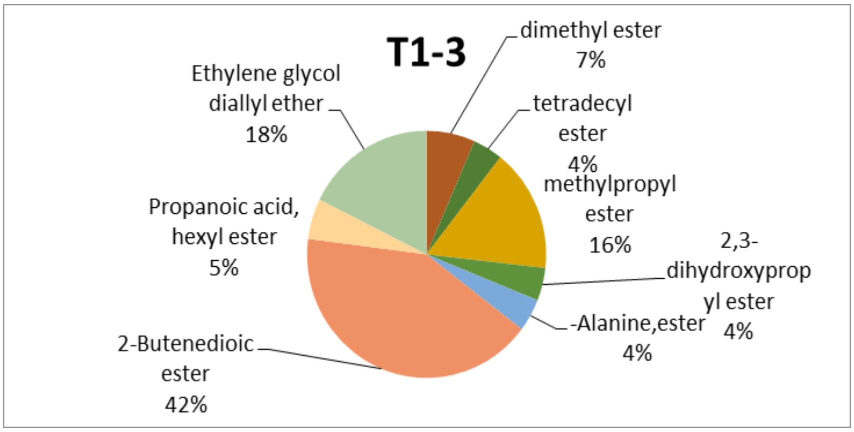

T3-3
Glycerol 1-palmitate 7%
1-Propanol, 2,2-dimethyl- 13%
-Indenol, 18%
Silanol, trimethyl 18%
1-Octanol, 2-butyl- 13%
Ethanol, 2-(dodecyloxy) 6%
1-Tricosanol 6%
Isopropyl Alcohol 12%
1-Undecanol 8%

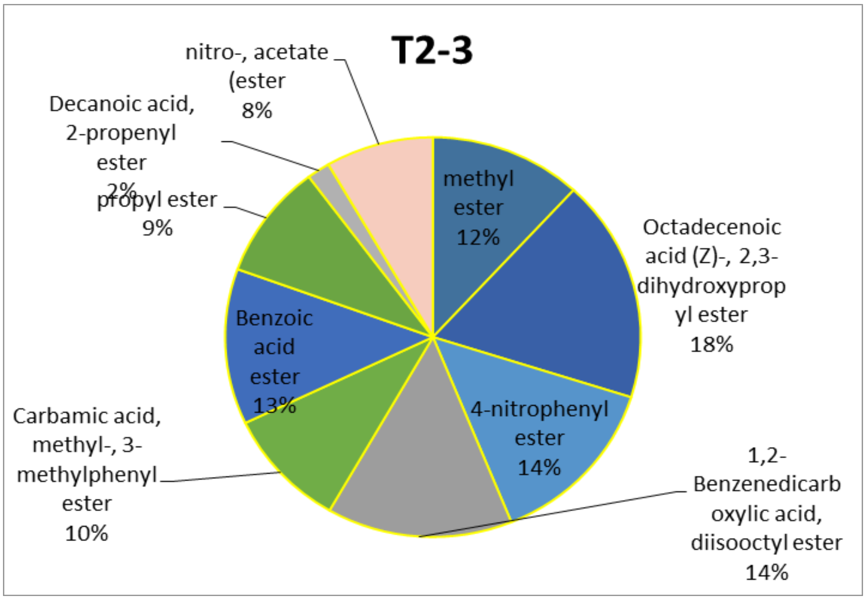
b-Aldehydes

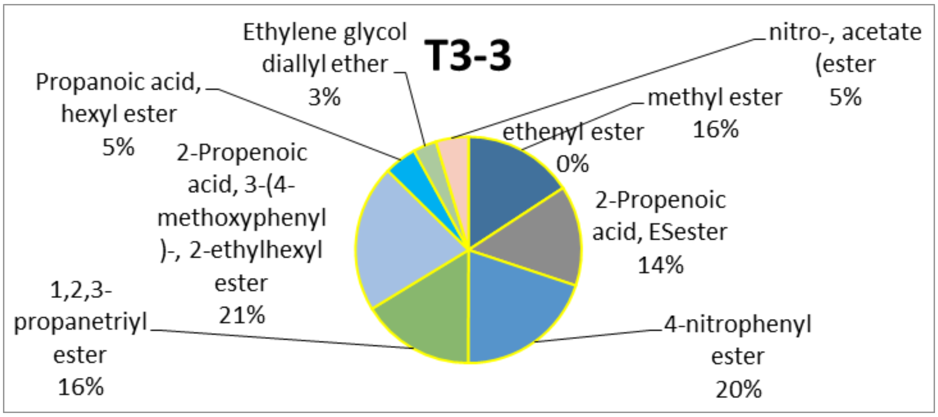


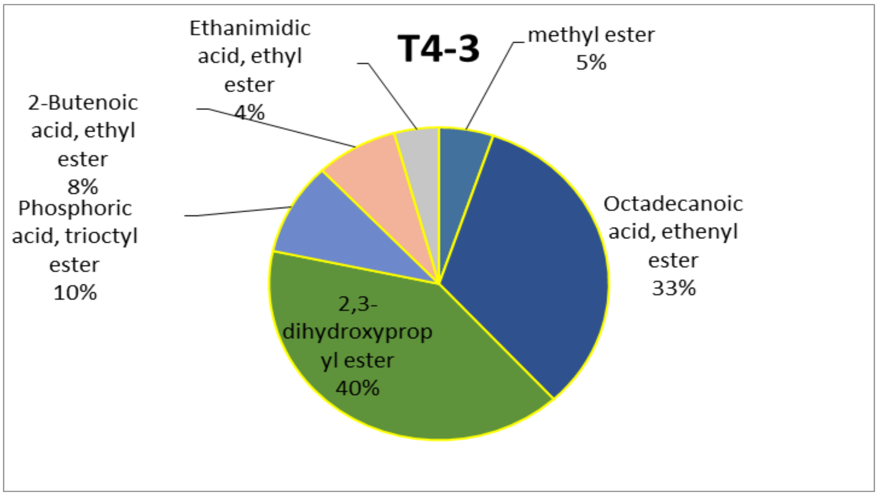
c-Esters



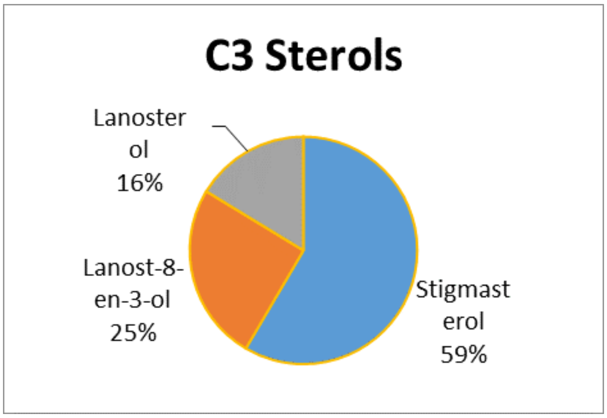


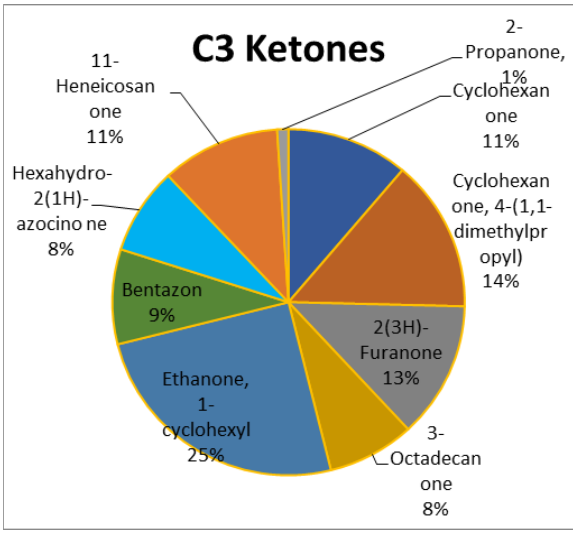

T2-3
nitro-, acetate (ester 8%
Decanoic acid, 2-propenyl ester 2%
propyl ester 9%
methyl ester 12%
Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester 18%
Benzoic acid ester 13%
4-nitrophenyl ester 14%
Carbamic acid, methyl-, 3-methylphenyl ester 10%
1,2-Benzenedicarboxylic acid, diisooctyl ester 14%

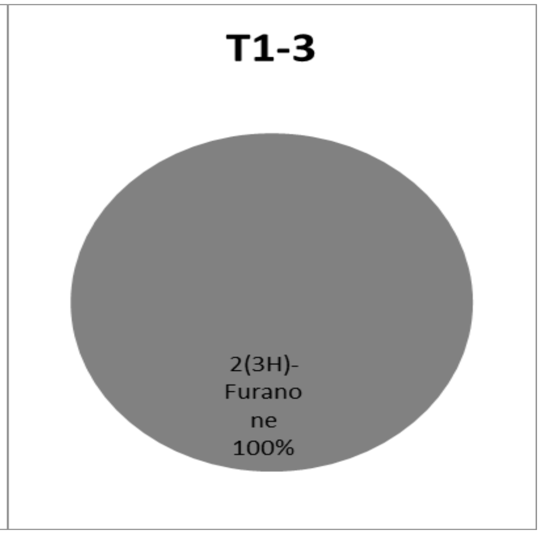

T3-3
Ethylene glycol diallyl ether 3%
nitro-, acetate (ester 5%
Propanoic acid, hexyl ester 5%
methyl ester 16%
ethenyl ester 0%
2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester 21%
2-Propenoic acid, ESester 14%
1,2,3-propanetriyl ester 16%
4-nitrophenyl ester 20%

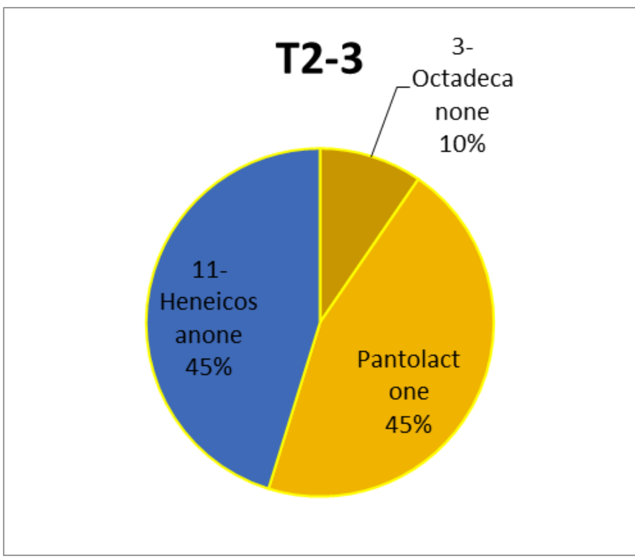

T4-3
Ethanimidic acid, ethyl ester 4%
methyl ester 5%
2-Butenoic acid, ethyl ester 8%
Phosphoric acid, trioctyl ester 10%
Octadecanoic acid, ethenyl ester 33%
2,3-dihydroxypropyl ester 40%

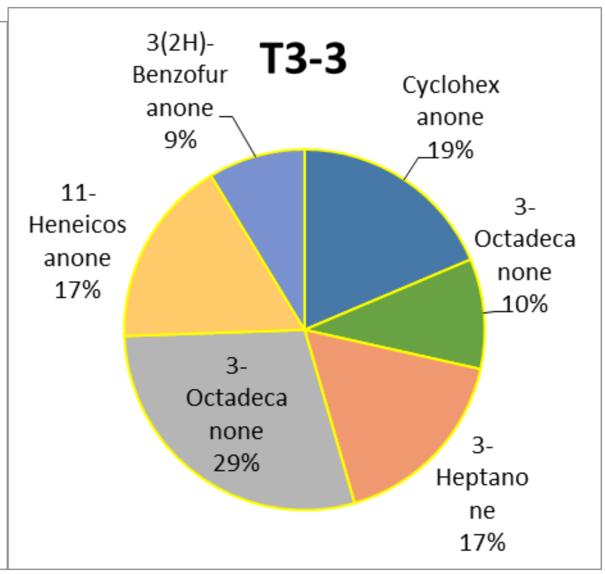
d-Sterols

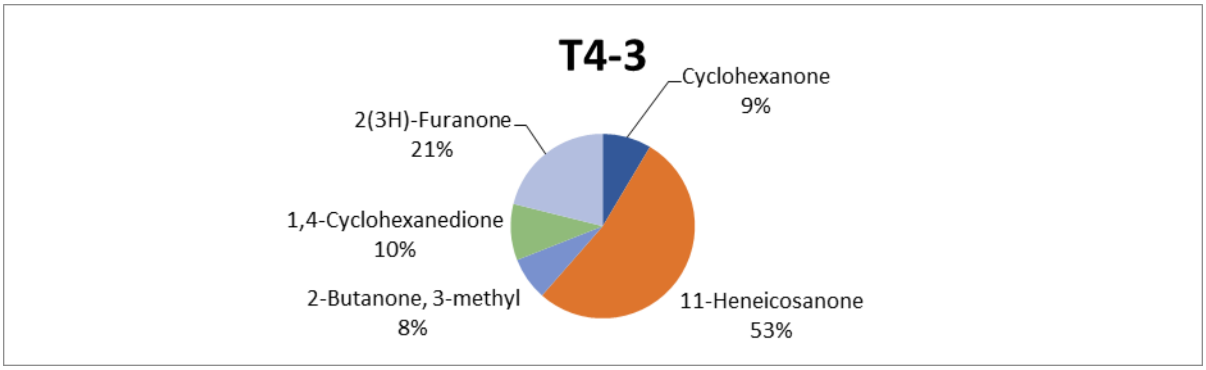

C3 Sterols
Lanosterol 16%
Lanost-8-en-3-ol 25%
Stigmasterol 59%


C3 Ketones
2-Propanone, 1%
Cyclohexanone 11%
Cyclohexanone, 4-(1,1-dimethylpropyl) 14%
2(3H)-Furanone 13%
3-Octadecanone 8%
Ethanone, 1-cyclohexyl 25%
Bentazon 9%
Hexahydro-2(1H)-azocino ne 8%
11-Heneicosanone 11%

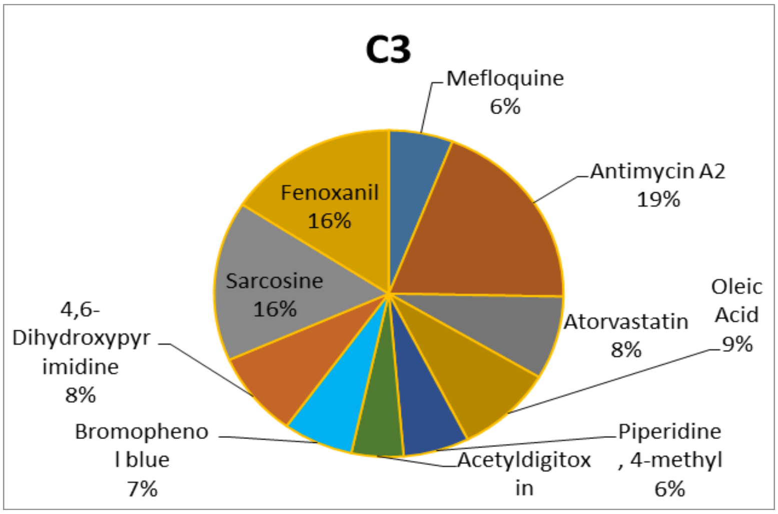

T1-3
2(3H)-Furanone 100%

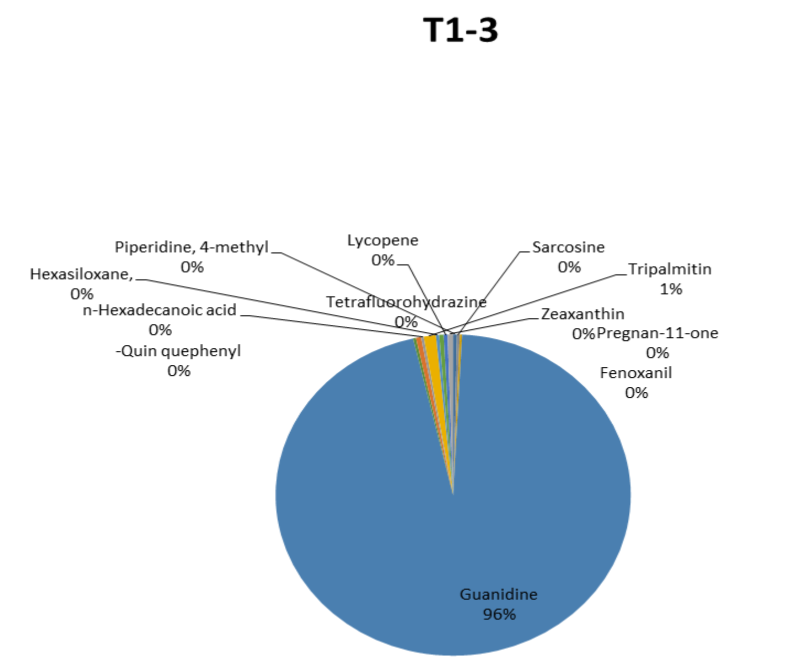

T2-3
3-Octadecanone 10%
11-Heneicosanone 45%
Pantolactone 45%


T3-3
3(2H)-Benzofuranone 9%
Cyclohexanone 19%
3-Octadecanone 10%
3-Heptanone 17%
3-Octadecanone 29%
11-Heneicosanone 17%



f-Other compounds





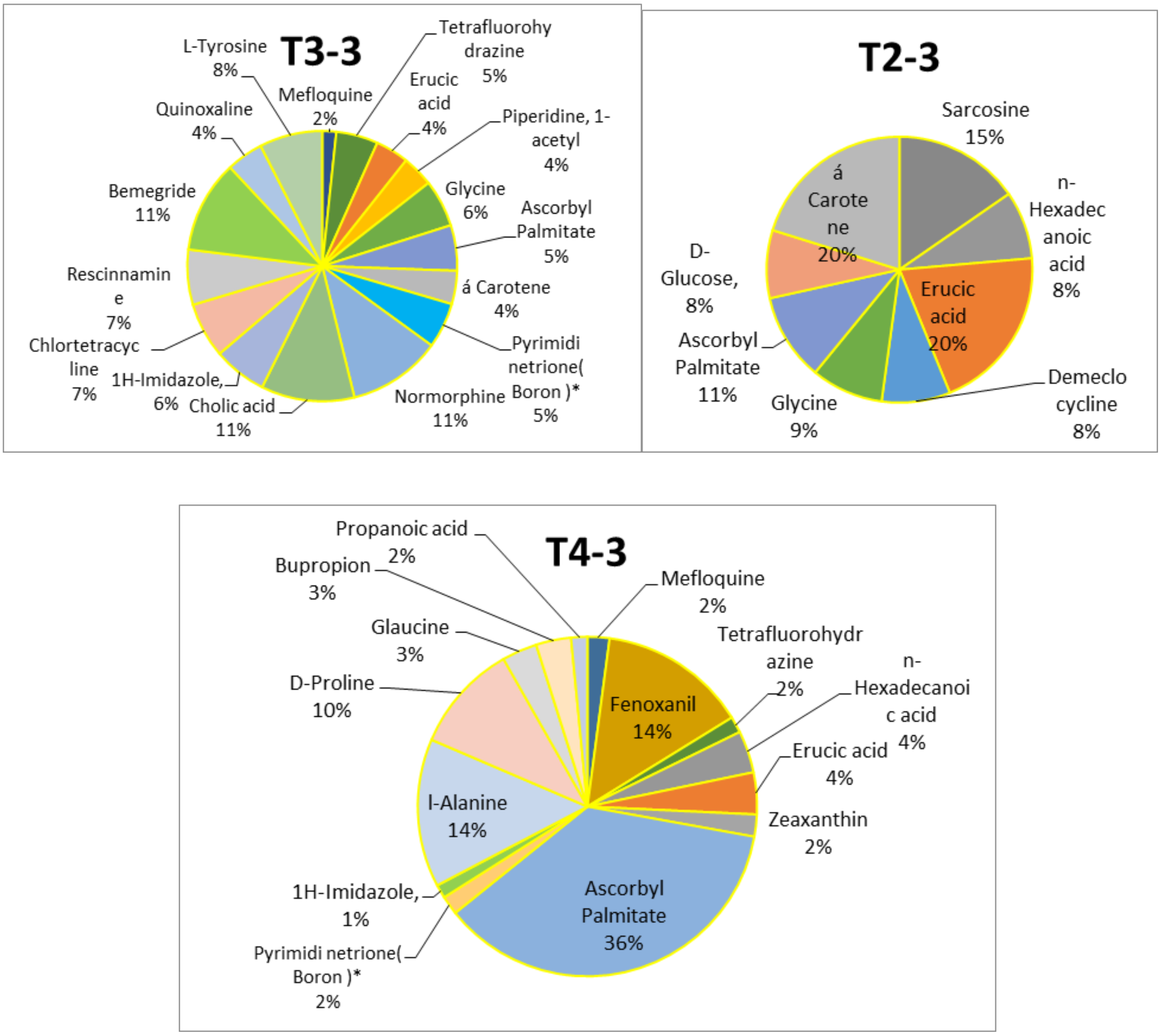


Fig. 3. Pie Charts of Volatile compounds by GC-MS analysis of of bio-stirred fortified yoghurt treatments after two weeks storage period

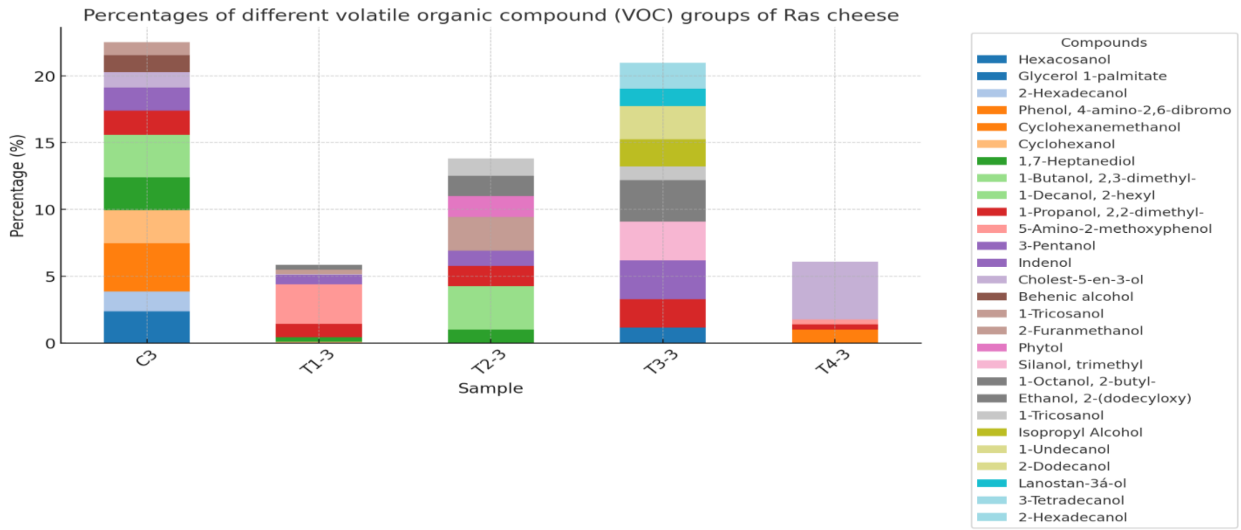


Fig. 4. The percentages of volatile organic compounds (VOCs) in the different samples (C3, T1-3, T2-3, T3-3, T4-3) using stacked bars

CONCLUSIONS

The outcomes of this Study is the better probiotic fortified yoghurt assess functionality and provides nutraceutical functions, enhancing functional promising metabolites, and successfully producing symbiotic. GC-MS methodology was a more comprehensive analysis for volatile compounds in bio-stirred fortified yogurt as well as the data analysis showed a strong positive correlation between the moringa percent and the bio-active polar and nonpolar compounds. The results also confirmed that combining in various ratios of adjunct cultures (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*) with moringa fortification supports and optimizes the growth, stability and health benefits of the final bio-product. Additionally the study ensuring that bioprocess technology of probiotic stirred-yoghurt maintained several biological functions, i.e. antifungals, anticancer, anti-inflammatory, and phytosteroid used in cardiopathies, also our findings highlight the potential of atorvastatin, the precursors of reductase inhibitor, it slowing cholesterol production which can be used as an ideal functional vulnerable diet to hypertension and blood pressure patients. Future efforts should focus on evaluating extended storage stability, conducting large-scale production trials for developing a new line of probiotic fermented dairy products and fermentation parameters that align with growing consumer demand for diet-based and functional foods.

ACKNOWLEDGMENTS

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الملخص العربي

التعرف علي المركبات العلاجية الوظيفية في الزبادي الحيوي المدعم بمستخلص المورينجا أوليفيرا

إنعام شكرى، سوسو العصفوري، إيمان ابراهيم

تزايد حديثا بشكل كبيراستهلاك الزبادي الحيوي ويفضل معظم المستهلكين تناول الزبادي الداعم للحيوية وذلك لطعمه المميز وقيمته الغذائية العالية ويهدف هذا البحث لتقدير مدي تأثير وصفات الجودة للزبادي المدعم بالمركبات المفيدة لصحة الإنسانولك من خلال تصنيع ٤ معاملات مدعمة بنسب مختلفة من المسحوق المجفف لأوراق وبذور المورنجا أوليفيرا مع معاملة كنترول للزبادي العادي بدون بادئ ثانوي مساعد وبدون التدعيم بمسوق المورنجا المجفف واستخدم في هذه الدراسة السلات الداعمة للحيوية من

Lactobbacillus acidophilus and *Bifidobacterium bifidium*

لتحضير منتج وظيفي وعلاجي مفيد لصحة الإنسان تم تصنيع زبادي مخفوق كوسيلة لتوصيل فوائد المورنجا والبادئ المساعد الحيوي للمستهلك. وتم تحليل GC-Mass لعينات الزبادي المدعم لتقدير المركبات الميتابولزمية الفعالة في كل من عينات الزبادي في بداية التصنيع وكذلك بعد فترة تخزين علي درجة حرارة الثلاجة لمدة أسبوعين لتقدير العمر التخزيني. وأوضحت نتائج الدراسة أن الزبادي الحيوي المدعم بمسحوق المورنجا بتركز ١% كان لها تأثيرات صحية وغذائية أفضل مقارنا بالتركيز الأقل من مسحوق المورنجا المجفف ٠,٥% حيث تعمل المورنجا كمكمل غذائي ومقوي للوصول للحالة الصحية المطلوبة للبادئ الثانوي. ووجد أن أضافة مسحوق المورنجا المجفف حسن من جودة وكفاءة المنتج النهائي ودوره الحيوي في الأمعاء وكذلك في التغذية العلاجية وتغذية الفئات الخاصة والحالات الصحية لمرضي القلب وضغط الدم المرتفع. ونحتاج للمزيد من الدراسات المستقبلية للتعرف علي الدور الحيوي المركبات الطيارة الناتجة من النشاط الأنزيمي والميتابولزمي للسلالات الداعمة للحيوية عند استخدامها مع المورنجا أوليفيرا.

الكلمات المفتاحية: المورينجا أوليفيرا، الزبادي الحيوي، السلالات الداعمة للحيوية، المركبات الميتابولزمية الفعالة، الزبادي الحيوي المُخفوق كمكمل غذائي.