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Identification and quantification of the main organic components in artisanal apple vinegars from Iraq Kurdistan region by 1H NMR spectroscopy

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ABSTRACT

The proton NMR spectra of apple vinegar samples obtained from the farms of Kurdistan region-Iraq**,** have been thoroughly examined and reported. Many organic molecules from various classes were assigned, including organic acids, alcohols, volatile compounds, and amino acids. Without extraction or pre-concentration processes, the possibility of quantifying the compounds that were present in the whole vinegar sample was also investigated. The results showed that ^{1}H NMR with water suppression allows a quick simultaneous determination of acetic, formic, lactic, malic, succinic, tartaric acids, ethanol, methanol, acetoin, 2,3-butanediol, glucose and fructose, by using dimethyl sulfone ($DMSO_2$) or potassium hydrogen phthalate (KHP) as internal standards. The ^{1}H NMR method was applied to differentiation of the various samples of apple vinegar.

1.Introduction

The usage of NMR in food research has grown significantly in the past 30 years (Gil et al., 1996), (Belloque and Ramos, 1999), (Andreotti et al., 2002), (Andreotti et al., 2000). There are many valid reasons for the substantial advancement in NMR technologies for food characterisation and regulation. In terms of sample preparation, analyte extraction and purification are typically not required. For instance, liquid food can be analyzed without any adjustments other than the addition of a deuterated solvent and an internal standard. No derivatization steps are needed for the detection of the analytes because any soluble molecules containing hydrogen can be detected by ^{1}H NMR. Another benefit of NMR over other analytical methods is the comparatively quick and simple data collecting process; a typical ^{1}H NMR spectrum can be obtained in just a few minutes. NMR is also the most powerful technique used to obtain structural information, and therefore it can facilitate the understanding of components' structure in complex food systems.

Due to these advantages, the NMR technique is very helpful for ensuring food quality. However, the development of NMR methods allows the quantitative analysis of food. In fact, the great development of the NMR application in food science was mainly concerned with the qualitative interpretation of the NMR spectra, and many examples can be found in the literature, including the characterization of tomato juice (Sobolev et al., 2003), tea (Le Gall et al., 2004) and wine (Pereira et al., 2005). Quantitative analysis by NMR has been less utilized (De las Heras et al., 2020) in food science, and only a few academic data are available on the validation of quantitative NMR (Nord et al., 2004), (Košir et al., 2001).

NMR can be advantageously used for quantitative analysis when certain technical and instrumental parameters are considered. One of the major advantages of quantitative analysis by NMR is the possibility of employing one internal standard for all the chemical substances. This is possible because the NMR response, under appropriate instrumental conditions, is precisely proportional to the number of nuclei (molecules) and can be considered the same for all the chemical components, including the internal standard. In light of this feature, ^{1}H NMR makes it possible to collect a lot of information and quantitative data in a single experiment.

Vinegar is a popular fermented culinary condiment all over the world, particularly in Asia and Europe. It was created and used as early as 3000 BC (Hemke et al., 2019), (Chen et al., 2013). Vinegar is used also extensively in healthcare including disease treatment (Perumpuli and Dilruksh, 2022). Research by (Budak et al., 2011) revealed that vinegar had a variety of pharmacological activities, including hypolipemia, antioxidant (Nagashima et al., 2010), antihypertensive (Nishikawa et al., 2001), and antidiabetic activity (Johnston et al., 2010). Nowadays, local vinegar is often made by small family-run businesses, following methods of production whose origins are to be found in ancient traditions. Vinegars are typically made in the Kurdistan area of Iraq from fruit like grapes, apples, and dates. Artisanal apple vinegar (AAV) are considered "special region vinegars". AAV is a very valuable product used for high quality gastronomy. Fabricated vinegar (FV) is a more common and cheaper product, having some organoleptic characteristics in common with AAV (colour, density, base taste, etc.).

The studies performed on vinegar in the past 30 years have shown that a full characterization of the product requires the determination of many classes of substances, e.g., carboxylic acids (Cocchi et al., 2002), (Plessi et al., 1989) alcohol (Gunduz et al., 2013), sugars (Masino et al., 2005), amino acids (Erbe and Brückner, 1998). Yet, the specific determination of the several vinegar components is a time-consuming procedure, which is not compatible with routine analysis. ^{1}H NMR spectroscopy allows for the simultaneous determination of the various vinegar compound groups in a short period of time (typically 10 minutes, including sample preparation and acquisition). This enables the examination of numerous samples, which is necessary for food authenticity and quality control.

Only few studies have been carried out on vinegars regarding their quality evaluation and

valorization. In researches (Caligiani et al., 2007), (Wang et al., 2016) the ^{1}H NMR spectroscopy was applied to investigated vinegars and quantitative results were provided.

In this paper the use of ^{1}H NMR for the simultaneous quantification of the main classes of artisanal apple vinegar components (carbohydrates, alcohols, organic acids, volatile compounds and amino acids) is shown. The ^{1}H NMR quantitative analysis was performed directly on the vinegar without any modifying of the samples, and the validity of the obtained data was studied.

2. Experimental

2.1. Materials

A total of 15 samples of apple vinegar were subjected to NMR analysis: 9 Artisanal apple vinegars (AAV); 6 Market apple vinegars (MAV). The artisanal vinegar samples came directly from three source producers, the vinegars were purchased from the market supposed to be artisanal, only one sample produce in factory again supposed to be 100% manufactured from apples FAV.

The reference samples for organic acids, sugars and derivatives, amino acids, alcohols, deuterated water, dimethyl sulfone $DMSO_2$ and potassium hydrogen phthalate KHP (internal standard reference for NMR analysis) were purchased from Sigma-Aldrich and other international companies.

2.2. 1H NMR analysis

Standard reference compounds were used; these were amino acids & derivatives (Hemke et al., 2019), small organic acids (Johnston et al., 2010), small organic alcohols, saccharides and sugar alcohols (Sobolev et al., 2003). These substances were measured by ^{1}H NMR. Table 1 shows the complete list of compounds. The signals were identified by recording ^{1}HNMR spectra of pure compounds, chosen among the major components present in vinegars (amino acids, organic acids, alcohols, sugars). A further confirmation of the signal assignments was obtained by spiking the vinegars with appropriate standards.

Both internal standards $DMSO_2$ and KHP were prepared first, KHP standard solution by weighing 0.1555g of KHP dissolved in D_2O 5.6503g (5ml) and for $DMSO_2$ standard solution by weighing 0.2084 of $DMSO_2$ dissolved in D_2O 11.0122g (5ml). The percentage of soluble substances was determined by ^{1}H NMR spectrometer, as a function of soluble substances, a variable quantity of the sample (220–380) mg (0.2ml) was weighed. About (120–140) mg 0.1mL of $DMSO_2$ or KHP standard solution was added to the vinegar weighed and the sample obtained was taken to 0.5 mL of final volume with deuterated water. For the spectra registered with water suppression, the solutions were placed in NMR sample tubes.

2.2.2. 1H NMR conditions

The spectra were recorded on a Bruker Spectrometer AVIII 400, operating at 14.1 T, equipped with a 5-mm triple resonance inverse probe with z-gradient. The ^{1}H NMR spectra were acquired both with and without low power selective water signal irradiation during 10000000000 s of the relaxation delay (d1). Data were collected acquired at 300 K with sample rotation at 20 Hz. 16 scans were acquired with a spectral width of 8333.333 Hz, an acquisition time of 3.9935999 s and a recycle delay of 5 s.

To better understand the characterization of vinegar, quantitative analysis of the assigned fermentation components was performed by integration using Amix 4.1.4 (Bruker Bio spins, Rheinstetten, Germany). Phase correction was performed manually for each spectrum, and the baseline correction was applied over the entire spectral range before FT transformation and standard integration routine. The concentrations of those components in prepared samples have been calculated based on the concentration of $DMSO_2$ or KHP that was added to each sample and proton numbers related to NMR signals of assigned components (Westwood et al., 2019).

3. Results and discussion

3.1. Signal identification

^{1}HNMR spectra of vinegar are characterized by a dominating water peak that is much greater than those of the components of interest. This causes a number of problems. Firstly, it prevents a correct digitization of small signals, hampering their observation and quantification; moreover, it makes it difficult to perform a correct quantitative analysis of the frequencies close to the water

signal tail. On the other hand, it was found(Boffo et al.,2009) that water suppression procedures can also modify the resonances near the water signal. So, the water suppression experimental parameters must be accurately calibrated to reach good suppression of the strong signal with the least perturbation of the closest frequencies.

Fig. 1 shows the ^{1}H NMR spectra, registered with water suppression, of a sample of vinegar, with expansions of characteristic zones. The signals were identified by recording NMR spectra of pure compounds, chosen among the major components present in vinegars (amino acids, organic acids, alcohols, sugars). A further confirmation of the signal assignments was obtained by spiking the vinegars with appropriate standards. The chemical shifts (especially of acidic and basic species) may be pH dependent, so spiking ensures that the component genuinely has the same chemical shift as the authentic reference species. However, it is still possible that coincident chemical shifts could arise for pairs of different compounds. Where possible ^{1}H NMR assignments should be confirmed by complementary techniques such as ^{13}C NMR and mass spectrometry.

The complete assignment of the signals identified and subsequently utilized for the quantification is reported in Table 1. Among organic acids, propanoic, acetic, lactic, malic, pyruvate, succinic, malonic and formic acids give signals which are well separated from each other and their quantification was thus possible. Tartaric acid is not always detectable in NMR spectra of vinegar, because in some cases it disappears with water suppression. Fig. 1.

2,3-Butanediol gave respectively one CH_3 signal which is well separated from the ethanol CH_3 signals, and so their quantification was possible Fig1,2,4&5. Also, ethyl acetate CH_3 for ethyl group can be detected and the signal quite separated from the ethanol CH_3, and so their quantification was possible. While, the overlap between the acetate signal of ethyl acetate and that of acetic acid will be overestimated when EtOAc is present as a minor component Fig 1,2&4. Acetoin gives a separate doublet at 1.295 ppm, but in some samples a slight overlap with lactic acid is observed; this could increase error in the acetoin quantification Fig1,2,4&5. Amino acids & derivatives are present only in some apple vinegars and at low concentrations: threonine and alanine were identifiable with methyl hydrogen signals as doublets at 1.23 & 1.40 ppm respectively Fig 2&4. *β*-alanine, and taurine with methylene hydrogen signals as triplets at 2.46 & 3.19 ppm.

The only sugars detectable in AV by ^{1}H NMR were mannitol, glucose and fructose, because all minor sugars are overlapped by their strong signals. The signals chosen for the quantification of glucose in vinegar is the C2H signal 3.153 ppm; for fructose the signals centered at 3.953 and 4.023 ppm were chosen, corresponding respectively to the C6H (axial position) and C5H hydrogens and for mannitol at 3.778 & 3.807 ppm belong to C1Hα and C6Hα. Glycerol give signals between 3.45-3.50 as multiplet for C1Hα + C3Hα Fig 1,2,4,5&6; again, in some samples the signals overlap slightly with minor sugars.

The quantification of a substance from an NMR spectrum requires knowledge of the number of hydrogens contributing to the signals of the analyte and the internal standard. The assignment of the mass fraction purity of an organic analyte A by qNMR in solution using an internal standard S is based on the equation given below (Amin and Claridge, 2017) & (Pauli et al., 2014).

$$w_{\mathrm{A}} = \frac{I_{\mathrm{A}}}{I_{\mathrm{S}}} * \frac{N_{\mathrm{S}}}{N_{\mathrm{A}}} * \frac{M_{\mathrm{A}}}{M_{\mathrm{S}}} * \frac{m_{\mathrm{S}}}{m_{\mathrm{A}}} * w_{\mathrm{S}}$$

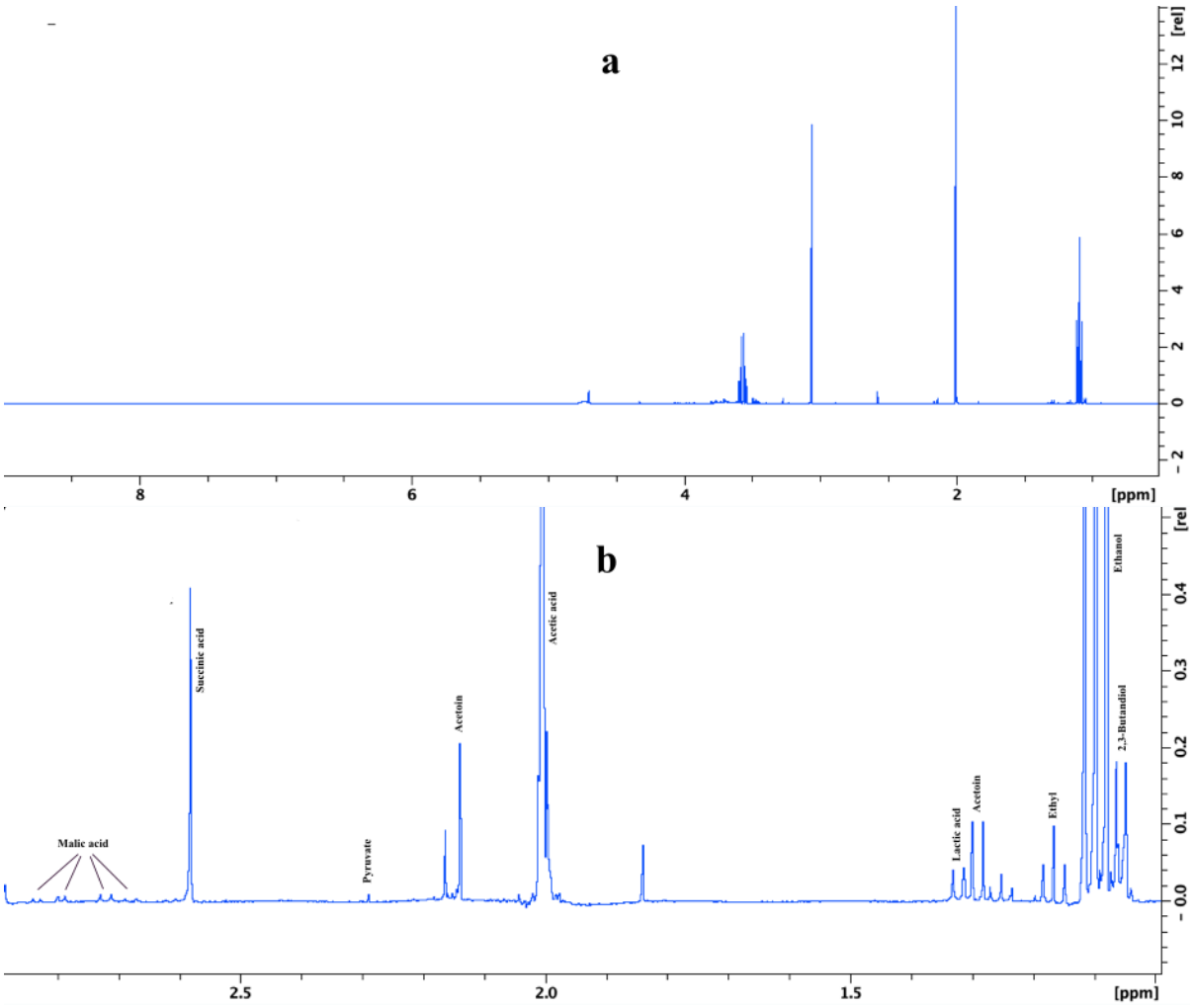
W_A is the mass fraction of the analyte in the material subject to assignment, W_S the independently established mass fraction of the internal standard, I_A and I_S are the integrals of the quantified signals, N_A and N_S the number of ^{1}H nuclei contributing to each quantified signal, M_A and M_S the molar masses of the analyte and internal standard and m_A and m_S the masses of the analyte and internal standard material used to prepare the solution subject to the qNMR measurement.

All AAV samples and one AMV sample show significant integration results for ethanol signals, and the opposite is observed for acetic acid. This may be due to not giving the samples enough

time to complete the fermentation or the temperature was lower than was required for fermentation to occur. As reported in (Consonni et al., 2004) the ethanol concentration was found to decrease with increase in the vinegar age. Although only one sample did not contain methanol, the concentration of methanol is below than the range of United States legal limit for fruit brandy (0.35% by volume or 280 mg/100 mL) (Woodams et al., 2010).as in table-2&3.

To check the consistency of the method, separate samples were prepared using the two different internal standards, dimethyl sulfone and potassium hydrogen phthalate. For the main components, including ethanol and acetic acid, there was generally good agreement (within 20% of the measured value) between results obtained with the different standards as in table-4. Minor components such as malic acid presented more of a challenge as the uncertainties in the measured integrals due to difficulties with phasing or sloping baselines were proportionately larger. Thus, although this method provides quantitative data for major components it should be regarded as providing more of a qualitative preliminary screen for minor species. Where appropriate these could be further investigated using more sensitive and specific analytical methods such as mass spectrometry and amino acid analysis to confirm presence of amino acid.

Table-5 showed, numerical result of the amino acid analysis and Fig.7 the chromatogram of the amino acid analysis results for AA8 sample. There is a lot of alanine in AA8, and smaller amounts of other standard amino acids, including possibly gamma-aminobutyric acid. Amm is just ammonia, which vinegar could have picked up anywhere, including from the lab.



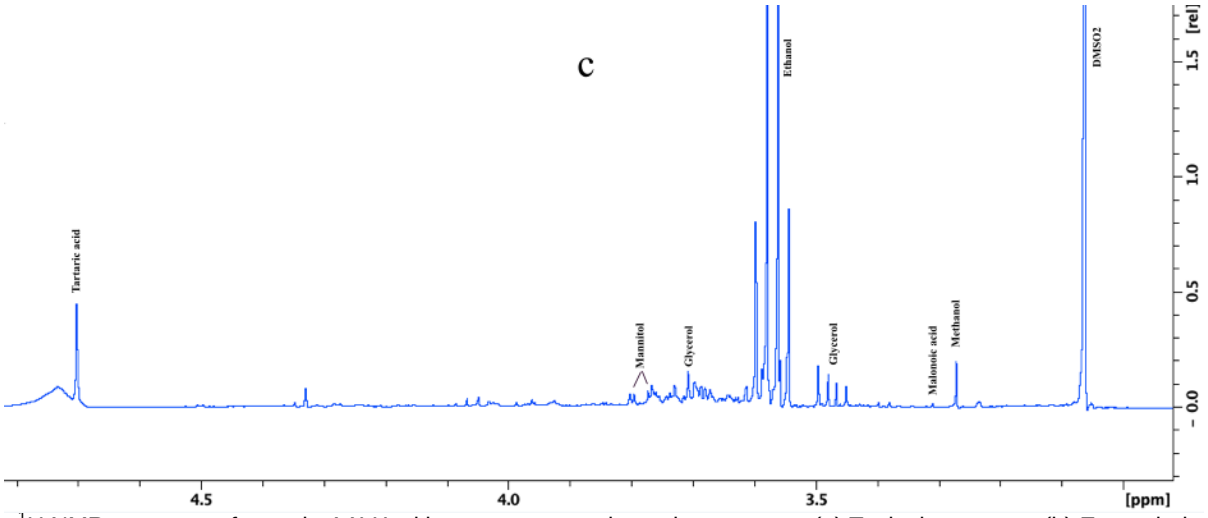


Figure1.[1]H NMR spectrum of sample AAV4 with water suppression pulse program. (a) Typical spectrum. (b) Expanded regions

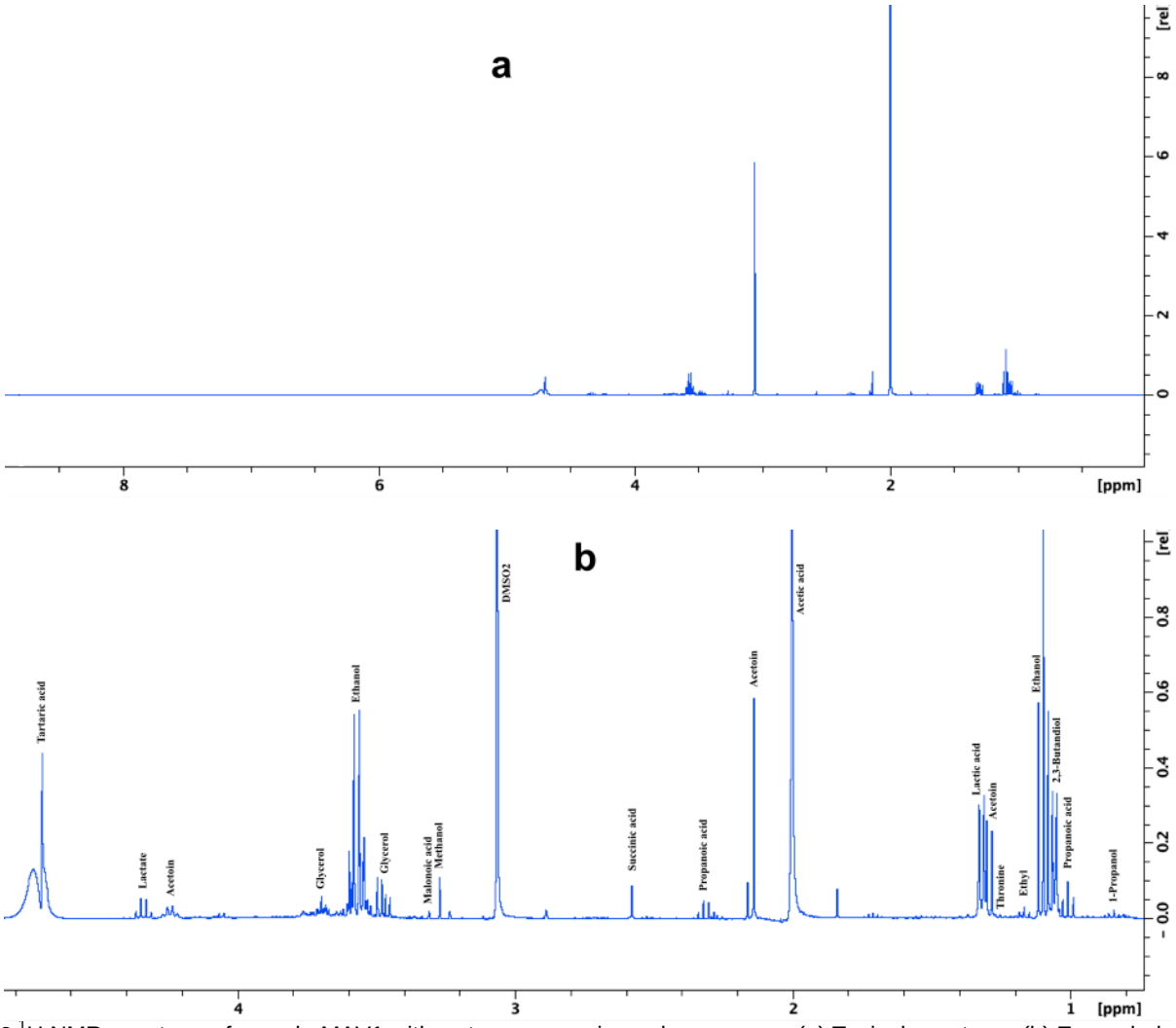


Figure 2.[1]H NMR spectrum of sample MAV1 with water suppression pulse program. (a) Typical spectrum. (b) Expanded regions

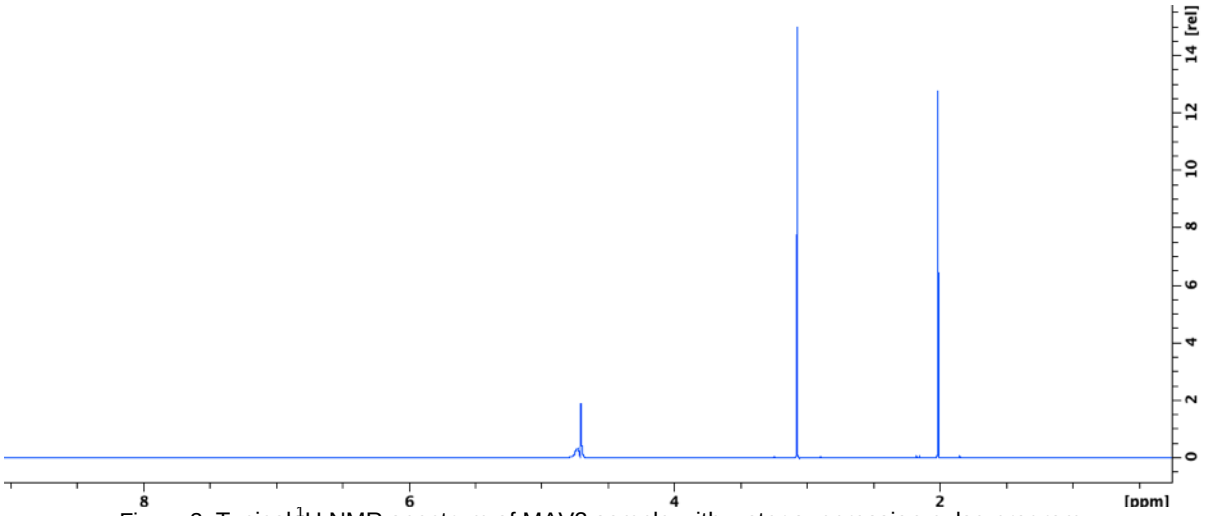
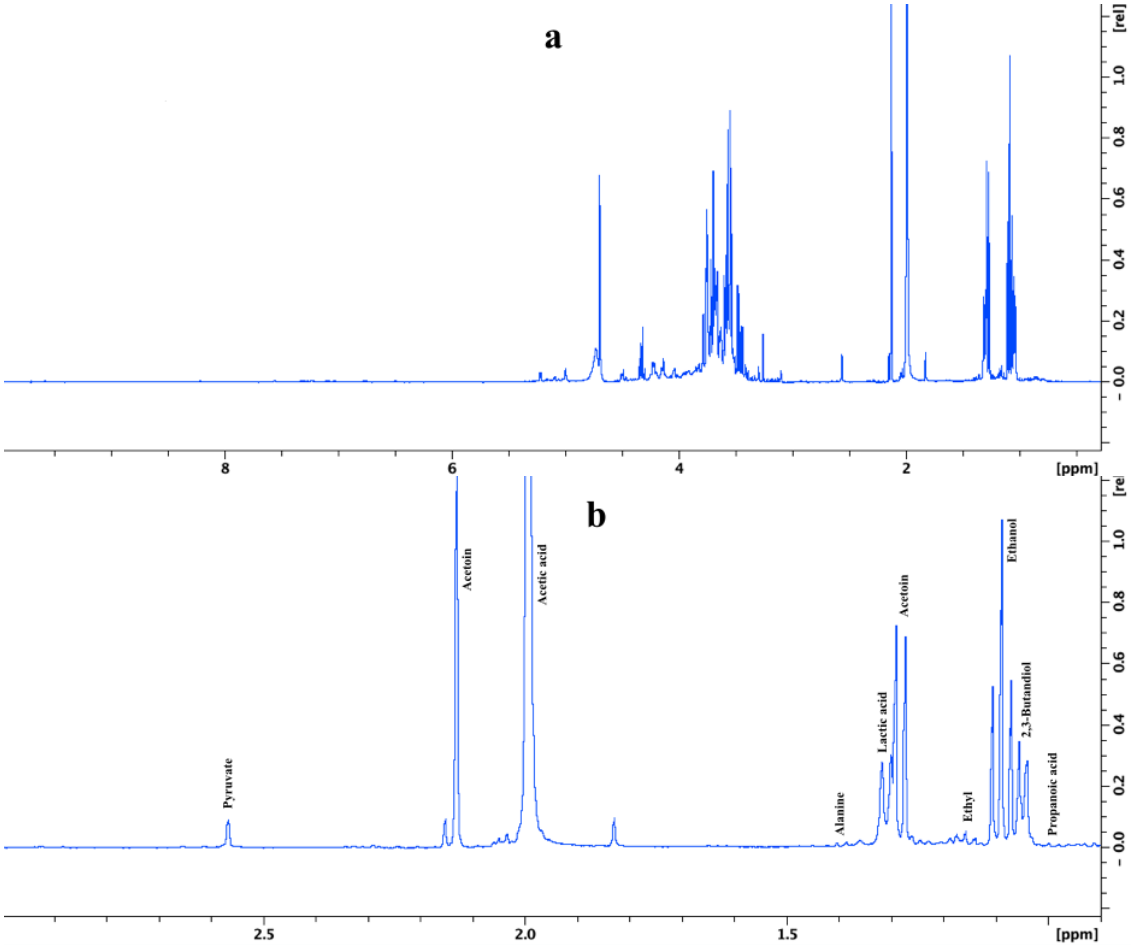


Figure 3. Typical ^{1}H NMR spectrum of MAV3 sample with water suppression pulse program.



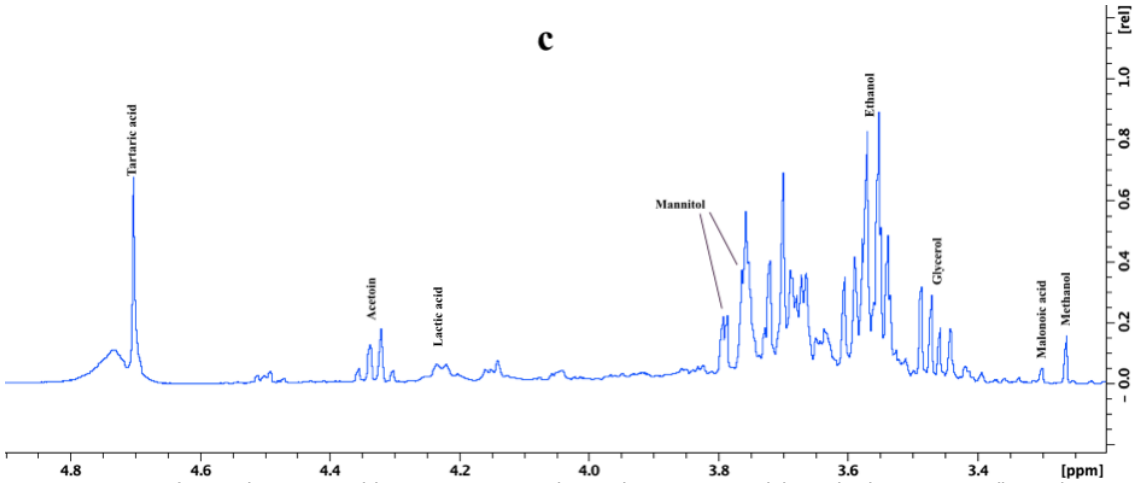
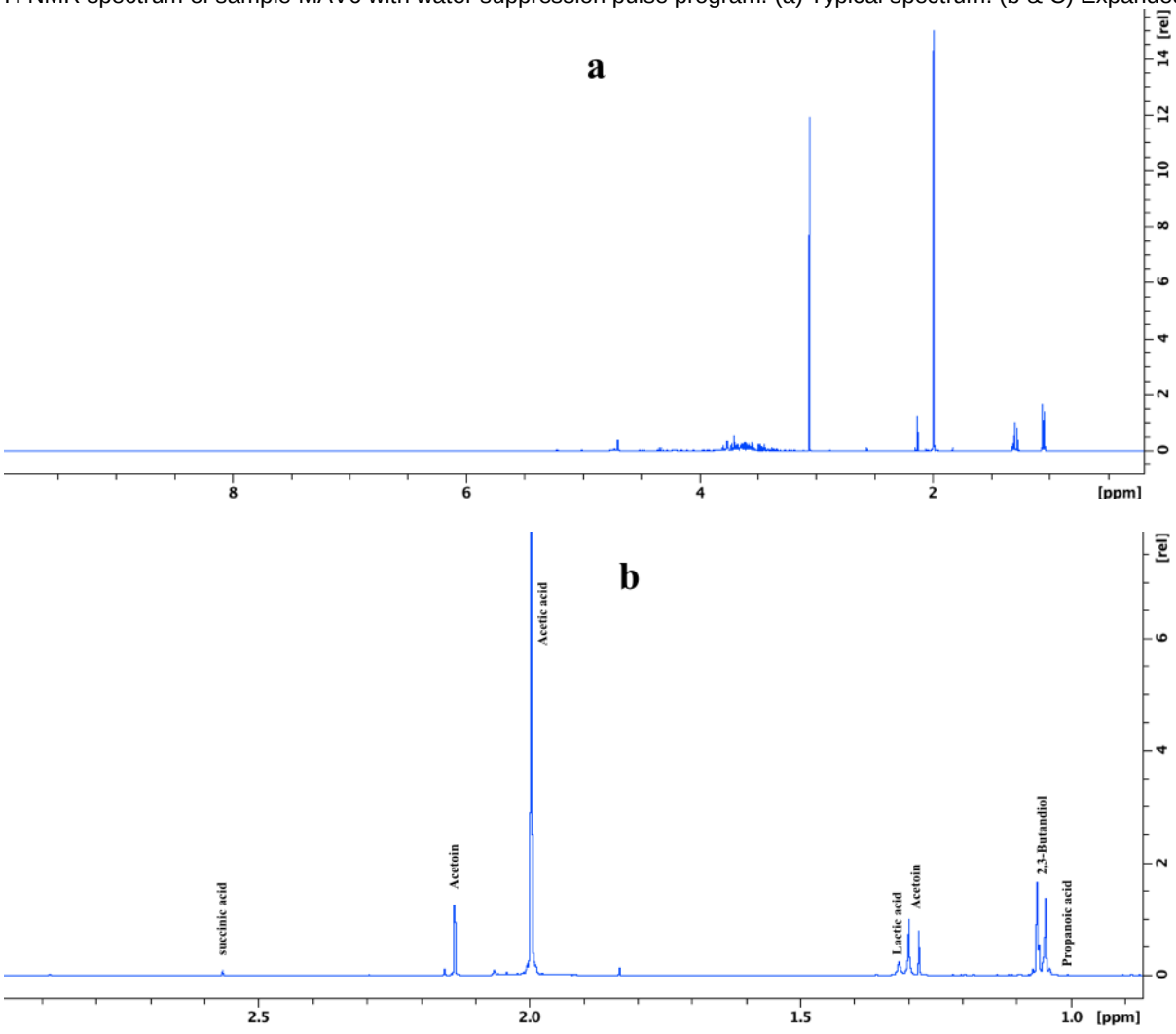


Figure 4.[1]H NMR spectrum of sample MAV6 with water suppression pulse program. (a) Typical spectrum. (b & C) Expanded regions



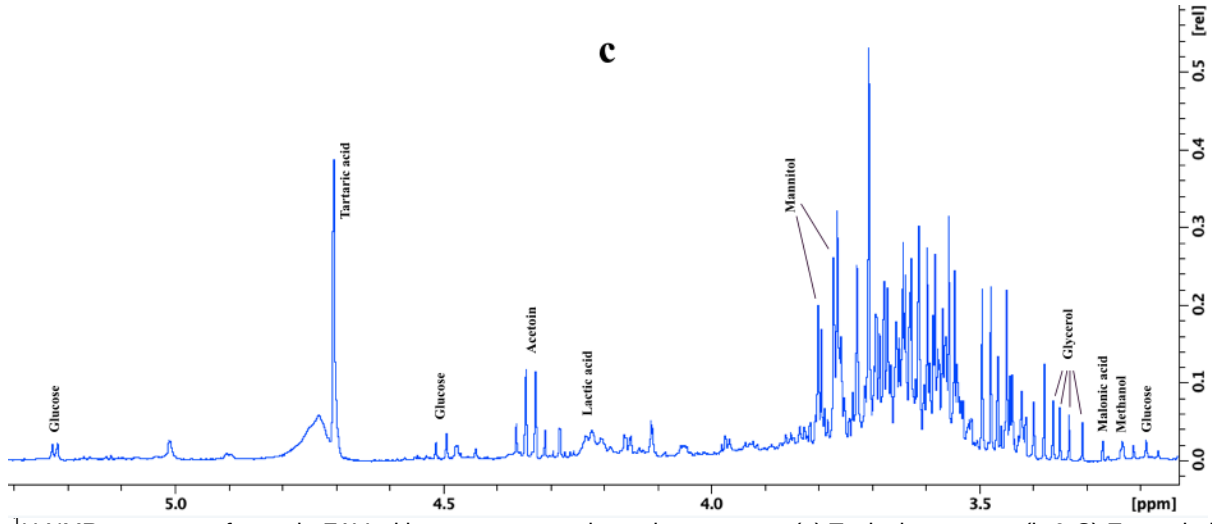


Figure 5. ^{1}H NMR spectrum of sample FAV with water suppression pulse program. (a) Typical spectrum. (b & C) Expanded regions

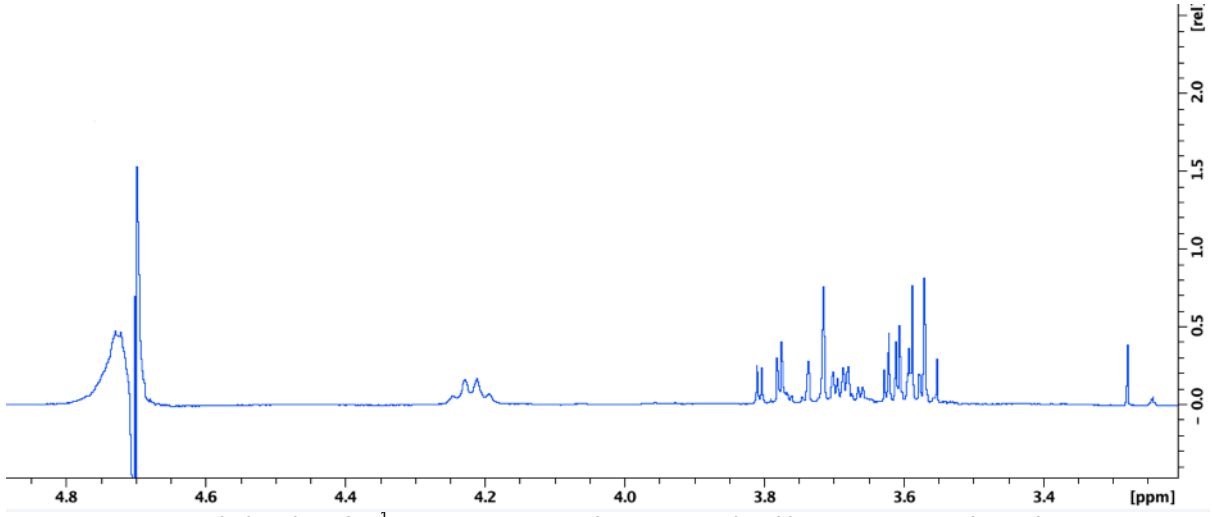


Figure 6. Expanded regions for ^{1}H NMR spectrum of AAV8 sample with water suppression pulse program.

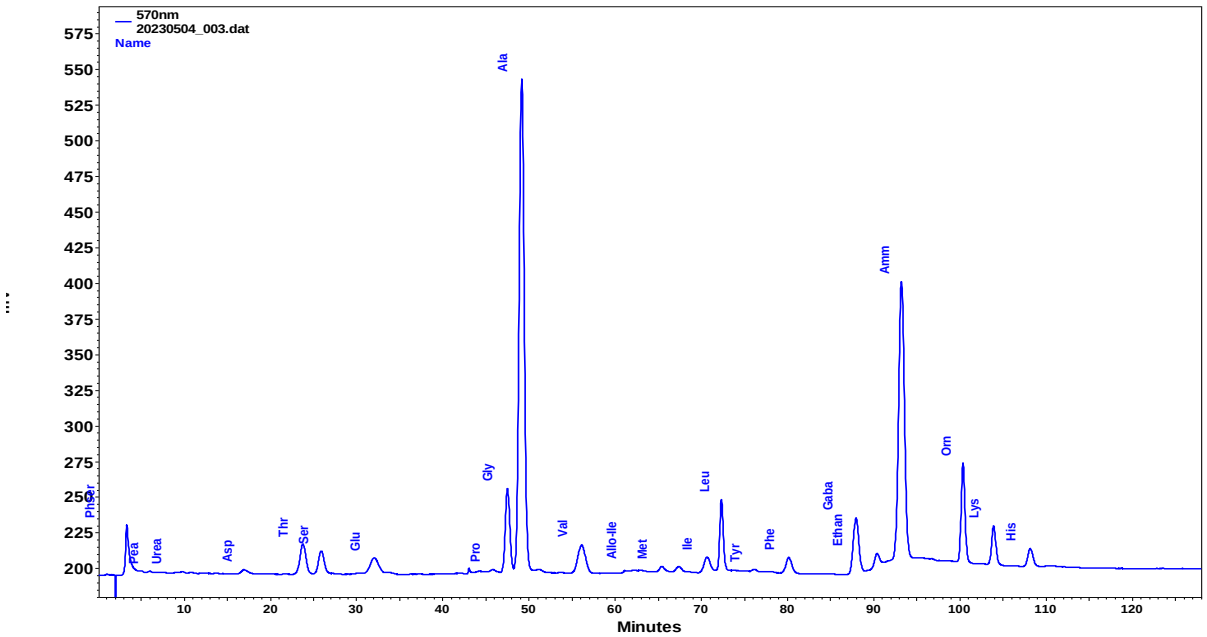


Fig.7 the chromatogram of the amino acid analysis results for AA8 sample.

Conclusion.

The key thing is that NMR can show all soluble organic components that are present in sufficient concentration. It should be recognised that some types of compounds (particularly carbohydrates) tend to give overlapping peaks which interfere with quantitative measurements, but even in these cases NMR provides a useful indication of what compound classes may be present and what further analyses may be appropriate. Finding ethanol in samples where consumers of the vinegar would not expect or want it seems to me to be a significant result that needs to be highlighted.

Acknowledgment.

Special thanks and appreciation to Dr. Peter B. Wyatt, Dr. Nasima Kanwal and Mr. Saeed Y. Yalda for helping me complete this research.

Table -1: Characteristics of 1H NMR signals observable in vinegar samples, used for quantitation of identified compounds.

Compound	Group	H No.	ppm	Multiplicity
1-Propanol	C_3H_3	3	0.84	t
Isoleucine	C_4CH_3	3	0.86	t
Leucine	$2\gamma CH_3$	6	0.87	dd
Valine	$2\delta CH_3$	6	0.93	dd
Propanoic Acid	C_3H_3	3	1.00	t
2-Propanol	$C_1H_3 + C_3H_3$	3	1.14	d
2,3-Butanediol	$C_1H_3 + C_4H_3$	6	1.06	d
Ethanol	C_2H_3	2	1.10	t
Ethyl	CH_3	3	1.17	t
Tert-Butyl Alcohol	$3\alpha CH_3$	9	1.14	s
Threonine	C_4H_3	3	1.24	d
Acetoin	C_4H_3	3	1.30	d
Lactate	C_3H_3	3	1.33	d
Alanine	C_3H_3	3	1.40	d
1,4-Butanediol	$C_2H_2 + C_3H_2$	6	1.45	s
Acetic acid	C_2H_3	3	2.01	s
γ-Amino Butric Acid	C_3H_2	2	1.71	P
Malic Acid	C_3Ha	1	2.72, 2.82	dd
Pyruvic Acid	C_3H_3	3	2.29	s
Ɓ-Alanine	C_2H_2	2	2.51	t
Succinic Acid	$C_2H_2 + C_3H_2$	4	2.59	s
Citric Acid	$C_2Ha + C_4Ha$	4	2.71, 2.85	dd
DMS stander	CH_3	6	3.07	s
Histidine	CH_{ring}	1	7.07	s
Glucose	C_5H_1	1	3.15	dd
Arginine	C_5H_2		3.18	t
Taurine	$C_1H_2 + C_2H_2$	2	3.17	t
Methanol	CH_3	3	3.27	s
Malonic acid	C_2H_2	2	3.31	s
1,5-Pentadiol	$C_1H_2 + C_5H_2$	4	3.51	t
1,2-Ethandiol	$C_1H_2 + C_2H_2$	4	3.53	s
Mannitol	$C_3H + C_4H$	3	3.77, 3.80	m
Glutamic Acid	C_2H_1	2	2.45	t
Fructose	C_6H		3.96, 4.03	dd
Glycerol	$C_1Ha + C_3Ha$	2	3.48	m
Glygolic Acid	C_2H_2	2	4.10	s
Tartaric Acid	$C_2H + C_3H$	2	4.70	s
Formic Acid	HC	1	8.17	s

Table-5 Numerical result of the amino acid analysis

AA	Standard, Area	Stand. uM	Sample AA8, Area	Result, uM	Comments
Phser	1181742501	500	111845925	47	Unlikely to be this material
Pea	1011085520	500	3422513	2	Unlikely to be this material
Taur	780233765	500			
Urea	399975225	5000	1355118	17	
Asp	1208700572	500	12811028	5	
Hypro	29718551	500			
Thr	1203532564	500	99780498	41	
Ser	1207263878	500	72807250	30	
Asn	853215489	500			
Glu	1237183029	500	67335417	27	
Gln		500			
Sarc	149821124	500			
AAAA	971478512	500			
Pro	64379061	500	4968012	39	
Gly	1248837159	500	215649802	86	
Ala	1178113034	500	1438199718	610	As expected
Citr	1254472403	500			
Aaba	1176938955	500			
Val	1113690801	500	122599485	55	
Cys	652150742	250			
Met	1210464220	500	14893132	6	
Allo-Ile	1114089474	500	15610200	7	
Ile	1142820839	500	52848466	23	
Leu	1244946979	500	145820176	59	
Nleu	1187686968	500			
Tyr	1209912067	500	5174748	2	
B-Ala	300167684	500			
Phe	1205078730	500	50477600	21	
Baiba	301938225	500			
Homocys	1038642100	500			
Gaba	919471707	500	172775484	94	Surprising result
Ethan	471840590	500	32744706	35	
Amm	863032453	500	924651320	536	
Hylys	1215496196	500			
Orn	1333816990	500	216052672	81	
Lys	1174790970	500	93305380	40	

pros-MeHis	1006367929	500			
His	1182446271	500	51391617	22	
Trp	815219791	500			
tele-MeHis	1083690916	500			
Ans	484985659	500			
Car	554312646	500			
Arg	1115828258	500			

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Table - 2: Concentrations (% *w/w*) of all analytes identified in vinegar samples from 1H NMR spectra data with water suppression using Dimethyl sulfone as internal standard reference

Compound	AAV1	AAV2	AAV3	AAV4	AAV5	AA6	AA7	AA8	MAV1	MAV2	MAV3	FAV	MAV4	MAV5	MAV6
Valine							0.0306	0.0071							
Leucine							0.0019	0.0066							
1-Propanol							0.0244		0.0140						
Propanoic Acid			0.0050				0.0041	0.0143	0.0647	0.0153		0.0222		0.0184	0.0168
2,3-Butandiol	0.1152	0.0489	0.0313	0.0874	0.0281	0.0171	0.0233	0.1524	0.1773	0.0915		0.5533	0.1126	0.2017	0.1295
Ethanol	1.5314	1.1777	0.0321	1.2180	1.9169	2.8189	0.1969	0.2491	0.2926	0.6525			2.7949	0.0230	0.2813
Ter-Butyl Alcohol						0.0009									
Ethyl	0.0111	0.0010	0.0039	0.0117		0.0078	0.0041		0.0108	0.0019			0.0500		0.0284
Threonine			0.0019				0.0147	0.0098	0.0181						
Acetoin	0.0941	0.1193	0.0136	0.0513	0.0060	0.0309	Not Int	0.0178	0.1832	0.0267		0.4554	0.0387	0.4504	0.3815
Lactate	0.1213	0.1978	0.0063	0.0363	0.0165	0.0146	1.0628	0.6238	0.3654	0.4330		Not Int	0.5764	0.2267	0.2354
Alanine					0.0199	0.0145	0.0239	0.0100					0.0086	0.0116	0.0081
1,4-Butandiol		0.0014													
Acetate	0.0657	0.0053	0.0425	0.3202	0.0078	0.0126							0.0989		
γ-Amino Butric Acid							0.0695	0.0346							
Acetic acid	1.3281	0.6626	3.5733	2.0138	0.3140	0.5565	0.1977	0.2536	2.9882	0.3673	1.0326	2.6653	1.6567	2.7078	3.3298
Pyruvate	0.0083	0.0083		0.0012	0.0046	0.0059			0.0021			0.0026		0.0035	
Malic acid	0.0891		0.1462	0.0099	0.1565	0.0895									
***Β*-Alanine**								0.0075							
Succinic Acid	0.0802	0.0326	0.0178	0.1012	0.0384	0.0357	0.0135	0.0377	0.0294	0.0314	0.0012	0.0195	0.0216	0.0192	0.0308
Glucose	Not D	0.3626	0.2698									0.1765			
Taurine		0.0628													
Methanol	0.0137	0.0050	0.0125	0.0196	0.0197	0.0208	0.0105	0.0126	0.0097	0.0088		0.0017	0.0466	0.0062	0.0134
Malonic acid	0.0187	0.0442		0.0079	0.0253	0.0310			0.0096	0.0037		0.0174	0.0353	0.0324	0.0282
Glycerol	0.2534		Not Int	0.2198	0.4667				0.2026	0.0995		0.1024	0.3684	Not Int	0.4776
Mannitol	0.0137			0.3943			0.6064	0.1087				Not Int	0.1903	0.8658	1.1974
Fructose		1.5787													
Tartaric Acid	0.6029	0.6215	0.4950	0.4893	0.4430	0.4693	Not Int	0.5953	1.1292	1.3431	1.9611	0.4501	0.4386	0.5371	0.6518
Formic Acid	0.0035	0.0018				0.0022									
Glycolic Acid						0.0633									

Table -3 Concentrations (% *w/w*) of all analytes identified in vinegar samples from 1H NMR spectra data with water suppression using Potassium hydrogen phthalate as internal standard reference

Compound	AAV1	AAV2	AAV3	AAV4	AAV5	AA6	AA7	AA8	MAV1	MAV2	MAV3	FAV	MAV4
Valine							0.0030	0.0048					
Leucine							0.0035	0.0049					
1-Propanol							0.0238		0.0103				
Propanoic Acid			0.0040				0.0084	0.0109	0.0415	0.0417		0.0142	
2,3-Butandiol	0.1010	0.0969	0.0311	0.0910	0.0316	0.0348	0.0237	0.0262	0.1599	0.0882	0.0011	0.5504	0.1200
Ethanol	1.4646	0.9820	0.0480	1.1807	2.1580	2.0385	0.1898	0.2112	0.2844	0.6651			2.7917
Ter-Butyl Alcohol													
Ethyl	0.0102	0.0101	0.0047	0.0161			0.0026		0.0111				0.0585
Threonine								0.0142	0.0142				
Acetoin	0.0822	0.1173	0.0125	0.0484		0.0278			0.1754	0.0185		0.4338	
Lactate	0.1063	0.1726	0.0054	0.0367	0.0130	0.0165	0.6879	0.7471	0.3297	0.4459		Not Int	0.5983
Alanine								0.0124					
1,4-Butandiol	0.0014	0.0018											
γ-Amino Butric Acid							0.0536	0.0585					
Acetic acid	1.3407	0.6452	3.7875	1.9817	0.3515	0.6293	0.1898	0.2039	2.7831	0.3165	1.0621	2.6892	1.7593
Pyruvate	0.0066	0.0090		Not Int	0.0061	0.0087							
Malate	0.0394		0.1700	0.0555	0.1729	0.1504	0.0117	0.0075					
Ɓ-Alanine								0.0174					
Succinic Acid	0.0707	0.0317	0.0202	0.1038	0.0476	0.0396	0.0115	0.0148	0.0324	0.0311	0.0013	0.0188	0.0264
Glucose	0.1139	0.4080	0.1802									0.1254	
Taurine													
Methanol	0.0137	0.0051	0.0134	0.0205	0.0240	0.0248	0.0141	0.0146	0.0100	0.0089		0.0023	0.0478
Malonic acid	Not Int			Not Int	0.0202	0.0483			0.0110	0.0041		0.0234	0.0364
Glycerol	Not Int		0.4000	0.2484	0.6171				0.1998	0.1101		0.1168	0.3994
Mannitol	Not Int		0.3234	0.5604			0.5058	0.3547				Not Int	0.2385
Fructose		1.8423											
Formic Acid	0.0012	0.0002				0.0048	0.0007	0.0008					
Glycolic Acid						0.0532							

Table - 4 Deference percentage of the two values for the concentrations of each main component in vinegar samples by using DMSO2 and KHP as internal standard reference.

Compound	AAV1%	AAV2%	AAV3%	AAV4%	AAV5%	AA6%	AA7%	AA8%	MAV1%	MAV2%	MAV3%	FAV%	MAV4%
1-Propanol							1.3711		15.0225				
Propanoic Acid			11.8082				-34.2936	13.5779	21.8136	-46.3103		21.8441	
2,3-Butandiol	6.5741	-32.9361	0.2195	-2.0157	-5.8574	-34.1226	-0.6705	70.6469	5.1632	1.8452		0.2607	-3.1807
Ethanol	2.2266	9.0615	-19.7762	1.5552	-5.9186	16.0662	1.8412	8.2339	1.4194	-0.9549			0.0575
Ter-Butyl Alcohol													
Ethyl	4.6470	-82.6716	-9.4360	-15.7653			23.0136		-1.6036				-7.8480
Threonine								-18.0422	12.0221				
Acetoin	6.7520	0.8359	4.2032	2.8127		5.3661			2.1625	18.1804		2.4290	
Lactate	6.5941	6.8146	7.3162	-0.6030	12.0666	-6.0844	21.4123	-8.9944	5.1285	-1.4710			-1.8630
Alanine								-11.0262					
1,4-Butandiol		-12.9197											
Acetic acid	-0.4727	1.3304	-2.9096	0.8029	-5.6321	-6.1358	2.0395	10.8665	3.5536	7.4273	-1.4093	-0.4454	-3.0040
Pyruvate	11.5923	-3.9670			-13.8742	-18.9800							
Malic acid	38.6581		-7.5205	-69.7997	-4.9751	-25.3972							
$\mathcal{B}$-Alanine								-39.5583					
Succinic Acid	6.3316	1.3699	-6.2420	-1.2649	-10.6474		8.2274	43.5923	-4.9333	0.5257	-5.0585	2.0509	-10.1137
Glucose		-5.8908	19.9228		Not Int							16.9363	
Taurine													
Methanol	-0.0617	-1.3356	-3.8220	-2.3174	-9.9360		-14.7447	-7.4149	-1.8810	-0.7177		-14.8089	-1.2683
Malonic acid					11.2535				-6.7397	-4.8282		-14.6915	-1.6429
Glycerol				-6.1015	-13.8825				0.6891	-5.0748		-6.5551	-4.0397
Mannitol				-17.3928			9.0415	-53.0947					-11.2406
Fructose		-7.7063											