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Aroma Analysis of Apple Juice: Influence of Salt Addition on Headspace Volatile Composition as Measured by Gas Chromatography and Corresponding Sensory Evaluations

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ABSTRACT

Sensory response to the aroma of a food depends on the composition and concentrations of the volatiles of the headspace. When analyzing the headspace composition by gas chromatography, salts are often added to the sample as a means of increasing the concentration of the aroma compounds in the vapor phase (e.g. enrichment of the vapor phase). This will only give a correct impression of the original aroma when all volatile components are affected equally. In this study on apple juice aroma, it is demonstrated that the degree of headspace enrichment resulting from salt addition is different for esters, aldehydes and alcohols, In our collection system, the average degree of enrichment at 40°C (described in terms of Relative Peak Areas—RPA) was greater than 4 for alcohols, between 1·75 and 3·50 for aldehydes and less than 1·75 for esters. How these differences can be useful when trying to identify gas chromatography peaks is also discussed.

Sensory evaluations showed that aroma response is changed when salt is added to the juice, resulting in an increased aroma intensity and off-aroma. Fruit aroma was not affected. A comparison of the sensory responses with headspace gas chromatographic measurements indicates that increase in off-aroma can be related to the increase in alcohol percentage in the headspace.

From these results it can be noted that, when attempting to correlate

sensory scores with aroma component concentrations as measured by headspace gas chromatography, it is most important that the test conditions utilized for each analysis correspond as closely as possible with each other.

INTRODUCTION

In studying the aroma properties and quality of food materials, headspace gas chromatography (GC) is one of the favored methods. Its value is due to the fact that the sample examined has, in theory, a similar composition to that sniffed during sensory evaluation and, further, is the same as that present over the product during consumption (sensed by both inhalation through the nose and volatilization in the mouth). Over the years, there has been interest in methods of increasing the sensitivity of the GC analysis. One such technique involves the addition of various salts to liquid food systems to increase the concentration of components in the headspace—and thus their ability to be detected in gas chromatographic analysis. The effect of adding salt (and other non-volatile solutes) to GC samples has been investigated in a number of studies, but generally only for a few compounds (Jennings, 1965; Nawar, 1966; Nelson & Hoff, 1968; Buttery *et al.*, 1969; Wientjes, 1968; Voilley *et al.*, 1977). Few researchers have commented that the increases in headspace concentrations observed with salt addition can be different for different compounds (Nawar, 1971; Friant & Suffet, 1979; Kieckbusch & King, 1979), and fewer still have commented on the consequences this could have on the sensory properties of the sample (Kepner *et al.*, 1964; Weurman, 1969). Kepner *et al.* (1964) did go so far as to note that when salt addition is used to enrich the headspace for GC analysis, the aroma present over the solution 'is no longer true', while Weurman (1969) postulated that, if the analysis procedure used alters the distribution of volatile components between the food and headspace, then the results obtained will not reflect the odour of the product. No data were presented to demonstrate these postulations.

Whilst headspace GC methods can be a valuable analytical aid, it is necessary to take into account any effects related to salt addition when relating headspace gas chromatography results to the aroma and general sensory properties of foods (and especially when attempting to correlate GC measurements with sensory evaluations). There are a number of

examples in the literature (e.g. Morgan & Day, 1965; Von Sydow *et al.*, 1970; Sapers *et al.*, 1977; Watada *et al.*, 1981) where the analysis of the aroma of food products has been undertaken by headspace GC with added salt without the authors making any comments as to the eventual effect that the salt addition may have had on the results and conclusions obtained.

In studying the effect of salt addition on headspace concentration, it has been usual to measure the air–liquid partition coefficients of model volatiles in salt-free and salt-saturated solutions, generally using NaCl or Na_2SO_4. The increase in headspace concentration with salt addition (for a fixed concentration of volatile in the liquid phase) is presumably related to reduction of the available solvent in the liquid phase resulting from the presence of the non-volatile solute (salt) (Voilley *et al.*, 1977; Nawar, 1971). Kieckbusch & King (1979) demonstrated (with sucrose addition to a series of acetates) that the air–liquid partition coefficient (K) increased sharply as the concentration of sucrose in solution increases. They showed, however, that this increase in K apparently results from the assumption that the volatile concentration in the liquid phase (calculated on a total water basis) remains constant as sucrose is added. If the volatile concentration in the liquid phase is calculated on the basis of available water instead of total water (i.e. water involved in sucrose hydration is considered as unavailable solvent), then K will remain constant as sucrose concentration increases. The current work was undertaken to investigate the effect of salt addition on the headspace composition as measured by GC and aroma sensory evaluations of apple juice.

MATERIALS AND METHODS

Apple juice and model solutions

Following a preliminary sensory test to ensure high quality, a number of cartons from a single production batch of commercial apple juice, which previously had been shown to have high sensory scores for fruit aroma and aroma intensity and a low off-aroma score (Poll, 1981), were purchased locally. These were blended together to make a homogeneous juice.

Model solutions containing a series of esters, aldehydes and alcohols at the 1 ppm level were also prepared. Components of the model solution

were chosen because they have been reported in apple juice and because they represent stepwise variations in carbon chain-length. Esters include ethyl acetate, ethyl butyrate, butyl acetate, butyl butyrate and hexyl acetate. Aldehydes include butanal, hexanal and *trans*-2-hexenal. Alcohols include ethanol (at 25 ppm), butanol, hexanol and octanol.

Sodium chloride in amounts of 0, 12·5, 25 or 37·5 g was added per 100 ml of apple juice or model solution during the headspace analysis procedure, or to apple juice for organoleptic evaluation.

Sensory analysis

Two trained judges conducted the sensory analysis for aroma of the apple juice samples. (From earlier studies of apple juice, it was concluded that evaluations by these two judges agreed well with each other, and with the results obtained from a larger judging panel, of which they were members.) Each sample was randomly presented a total of four times to each judge, giving a total of eight evaluations. The samples were judged for aroma intensity, fruit aroma and off-aroma, on a scale ranging from 0 to 10 (10 = highest level for each characteristic). An analysis of variance test of the eight evaluations was conducted to determine which scores were statistically different at the 95% level.

Gas chromatographic analysis

The aroma components of the apple juice were collected and concentrated using a 'gas stripping with subsequent trapping' procedure. A 250-ml gas washing bottle containing 100 ml of sample and 0·3 ppm heptanol (internal standard) was placed in a 40 °C water bath over a magnetic stirrer and the desired amount of sodium chloride was added. After 20 minutes' equilibration, nitrogen gas at 60 ml min^{-1} was bubbled through the flask, exiting through a glass tube (11 × 0·4 cm) containing 300 mg Porapak Q (50–80 mesh). After 1 h, the Porapak tube was removed and the retained volatiles eluted with double-distilled ether. After collecting 160 mg ether in a $\frac{1}{2}$ ml V-bottom vial (Weaton Glass), the eluate was concentrated to 15 mg by gently blowing nitrogen gas over the ether surface. Of this concentrate, 0·5 μl was injected into the gas chromatograph (Hewlett-Packard HP-7620 Research Chromatograph with HP-33880 integrator). The GC separation was conducted using a 5 m × 3 mm stainless steel column packed with 10% OV-351 on

Gaschrom W, AW (80–100 mesh) with a nitrogen gas flow of 20 ml min^{-1} and the following temperature programme: 10 min at 70 °C, 70–170 °C at 4 °C min^{-1}, 170 °C isothermal. The injection port temperature was 200 °C, while FID temperature was 230 °C.

Each sample was analyzed in duplicate. A preliminary test conducted on four identical samples showed that the standard deviation as a per cent of the peak area, averaged for all GC peaks, was 15·0 % (ranging from 1·0 to 20·9 %).

Relative peak area values

The gas chromatography peak areas, as measured by the integrator, were subjected to a two-step correction procedure. For each injection, measured absolute peak areas were adjusted for small variations associated with the collection, trapping, extraction, concentration and injection steps by dividing each peak area obtained for that injection by the area of the internal standard for the same injection. To obtain the final 'corrected peak area', these adjusted peak areas were then multiplied by the averaged area for the internal standard for the two injections for that particular salt-added level. These final 'corrected peak areas' are thus again expressed on an absolute peak area basis. The ratio of the corrected peak areas for each volatile component for the sample containing 37·5 g salt to the corrected peak areas for the corresponding peaks for the sample without salt is called the Relative Peak Area of that component (RPA). As the concentration in the original liquid phase is the same for both samples prior to the addition of salt, the difference in peak area is directly a result of the headspace increase resulting from the addition of the salt.

RESULTS AND DISCUSSION

Influence of salt concentration in solution on headspace volatile concentration

The results of the gas chromatographic (GC) analysis for apple juice are given in Table 1. The values shown are the final corrected peak areas (adjusted for variation of internal standard area and then corrected back to an absolute area basis) at the four levels of salt addition. In addition,

TABLE 1

Gas Chromatographic Peak Areas (Corrected for Internal Standard) for Apple Juice Samples Containing Various Levels of Added NaCl and the Calculated Relative Peak Area (RPA) Values for Apple Juice from Model System Measurements

Peak number		*Corrected peak area*				*RPA*[b] *apple juice*	*RPA*[b] *model solution*
		0[a]	*12·5*[a]	*25*[a]	*37·5*[a]		
2	Ethyl acetate	459	574	607	800	1·74	2·18
3	Ester	11·3	13·5	14·1	18·2	1·61	
4	Ethanol	53·8	77·0	139	314	5·83	6·00
5	Ethyl propionate	67·1	74·2	77·4	92·4	1·38	
5a	Propyl acetate	33·5	38·8	40·5	50·2	1·50	
6	Methyl butyrate	15·3	16·9	16·3	19·6	1·28	
7	*Iso*-butyl acetate	20·7	20·9	20·2	26·2	1·27	
8	Ethyl butyrate	170	177	168	207	1·22	1·59
9	Ethyl-2-methyl butyrate	20·4	17·7	15·8	18·2	0·89	
10	Butyl acetate	728	791	822	962	1·32	1·75
11	Hexanal	66·6	69·5	—	174	2·61	1·69
11a	Alcohol	3·5	6·0	9·7	17·5	5·00	
12	*Iso*-pentyl acetate	104	94·8	84·9	98·3	0·95	
13	Butanol	255	387	618	1 223	4·80	6·39
13a	Pentyl acetate	9·2	8·4	8·8	10·9	1·18	
14	*Iso*-pentyl alcohol	79·4	131	224	426	5·36	
16	*Trans*-2-hexanal	316	409	522	803	2·54	3·51
17	Aldehyde	14·1	16·6	22·9	37·8	2·68	
18	Hexyl acetate	167	142	136	154	0·92	1·37
19	Ester (aldehyde)	5·8	6·2	7·9	9·5	1·64	
19a	Ester	50·2	52·8	53·6	61·1	1·22	
20	Hexanol	367	667	1 396	2 483	6·77	9·32
21	*Cis*-3-hexanol	3·3	7·2	15·8	40·0	12·1	
22	*Trans*-2-hexanol	27·3	47·0	103	218	7·97	
23	Hexyl butyrate	4·3	2·6	4·8	8·0	1·86	
24	Aldehyde	6·4	10·2	15·4	18·2	2·84	

[a] Grams of salt added per 100 ml of juice.

[b] Relative Peak Area (see 'Materials and Methods' section).

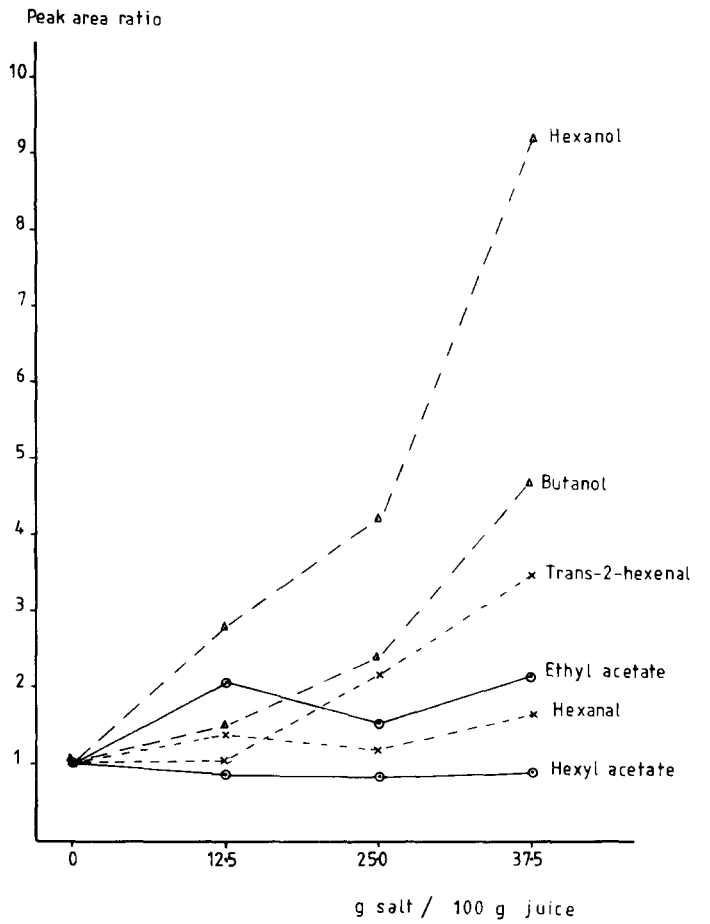

Fig. 1. Maximum and minimum peak area ratios observed for alcohols, aldehydes and esters at different levels of NaCl added to apple juice. (Peak area ratio at 37·5 g salt added = RPA value.)

there are columns giving the Relative Peak Area values (RPA = ratio of the peak area at 37·5 g salt to peak area without salt) for apple juice, and, in the applicable cases, RPA values found for the model solution. In addition, it can be noted that three components in the model solution, not found in this apple juice, had the following RPA values: butanal (2·07), butyl butyrate (1·30) and octanol (6·43).

Figure 1 presents the range of peak area ratios (highest and lowest) found for the esters, aldehydes and alcohols examined in this study at the various levels of salt addition. It can be seen that the three groups of

TABLE 2
Influence of Carbon Chain Length and Chain Branching on RPA Values for Acetates

	Relative peak area	
	Apple juice	*Model system*
Ethyl acetate	1·74	2·18
Propyl acetate	1·50	
Butyl acetate	1·32	1·75
Pentyl acetate	1·18	
Hexyl acetate	0·92	1·37
Iso-butyl acetate	1·27	
Iso-pentyl acetate	0·95	

compounds each have different sensitivity for change in peak area resulting from addition of salt to the solution. In particular, the peak areas for esters do not change very much with addition of salt, whereas the peak areas for alcohols show a very marked change. Aldehydes lie between these two groups.

An examination of data for a series of acetates (Table 2) indicates that the RPA values are dependent on carbon chain length, with the RPA value decreasing as the total chain length increases. Similar behavior was found for a series of ethyl esters. The observation of decreasing RPA value with increasing chain length is most probably due to increasingly non-ideal solution behavior in water as chain length increases. It has been reported (for salt-free solutions) that the volatility of a homologous series of compounds increases with increasing chain length (Buttery *et al.*, 1969; Kieckbusch & King, 1979). The volatility increase with increasing chain length can be expected to be lower for salt-saturated solutions, due to the non-ideal behavior already associated with the presence of the salt in the liquid phase. As RPA is defined as the ratio of headspace concentration with salt addition to the headspace concentration without salt addition, it can be observed that, as chain length increases, there is a larger volatility increase for the denominator (i.e. headspace without salt) and a lesser volatility increase for the numerator (i.e. headspace with salt) resulting in lower RPA values. Kieckbusch & King (1979) gave data which, when recalculated as RPA values, also demonstrated that the RPA value falls with increasing volatile chain length when sugar is present in aqueous solutions.

RPA measurements for the model solution showed similar decreases with increasing chain length, although the values are about 0·44 higher than for apple juice (Table 2). This higher RPA value could result from the presence of non-salt solutes (primarily sugars) in the apple juice. As noted above, Kieckbusch & King (1979) gave data which, when recalculated as RPA values, demonstrated that sugars in solution behave similarly to salt. In samples at high salt concentrations, sugars (in the apple juice) would have little extra effect on peak areas, whereas, in samples without salt added, sugars in the apple juice would increase the peak area for the apple juice samples relative to the model solution (which does not contain sugars). This will have the effect of reducing the RPA values for the apple juice samples.

Table 2 also contains RPA values for two branched compounds. In both cases, it was noted that the branched compound appears to have a slightly lower RPA value than the corresponding normal compound, although it must be emphasized that the differences noted are not statistically significant.

Possibility of using salt addition with headspace chromatography as an aid in identification of gas chromatography peaks

The observations presented above regarding the difference in RPA values for esters, aldehydes and alcohols gave rise to the idea that salt addition to solutions could be useful as an aid in the identification of GC peaks with headspace analysis. It should immediately be emphasized that the proposed method is suitable only as an aid in identification, and that eventual identification should be based on other identification techniques, such as retention time correlations, etc.

The proposed procedure involves preparing headspace chromatograms for samples (1) without salt added and (2) with salt added at the saturation level. The peak areas are calculated and the RPA values determined. Control of temperature during the calibration steps (i.e. with known compounds) and in examination of samples is very important. In a small, supplementary experiment conducted at 20 °C, it was observed that RPA values at 20 °C were 2–3·5 times greater than at 40 °C. Thus, it is important to realize that the actual RPA values to be used as an identification aid will depend on the headspace equilibration temperature, and that the RPA values mentioned here are valid for our collection system at 40 °C.

From Fig. 1 (or Table 1), it can be seen that, for salt additions at a level of 37·5 g NaCl/100 ml solution, the RPA values at 40 °C for esters ranged from 0·92 to 2·18, for aldehydes from 1·69 to 3·51 and for alcohols from 4·80 to 9·32. Whilst there can be some possibility of overlapping between esters and aldehydes, most compounds evaluated would be properly described for our analysis system by using the following RPA values to aid in the identification of GC peaks of apple juice:

Esters	$RPA < 1{\cdot}75$
Aldehydes	$1{\cdot}75 < RPA < 3{\cdot}50$
Alcohols	$RPA > 4{\cdot}00$

The identification of peaks listed in Table 1 was done on the basis of retention time data. On the basis of the above criteria, it can be noted that in essentially all cases where peaks have been identified by retention time, the RPA values verify these identifications.

There are a few exceptions to this, one of which is peak 23, identified by retention time to be hexyl butyrate. The observations with this peak can serve as an example of a potential source of error which can arise when applying the Relative Peak Area method to very small peaks on the chromatogram, as calculation of the RPA value will involve dividing by areas for small peaks which themselves can have sizeable standard deviations. Thus hexyl butyrate, with an RPA value of 1·86, could have been considered to be an aldehyde. This cases demonstrates that it must be the retention time data which are used for identification, with the RPA values serving as an aid in choosing which compounds to test. (If the RPA value had been 2·5, though, one could question if peak 23 might not be an aldehyde with the same retention time as hexyl butyrate.) Another special example is peak 21 (*cis*-3-hexenol), which shows an RPA value of 12·1, much higher than noted for any other alcohol. This most probably arises from the small peak area for the sample without salt addition.

In addition to the peaks which have been identified by retention times, there are six peaks in Table 1 which have not been identified by retention times using the pure standards available. On the basis of the above RPA criteria, peak 3 (RPA = 1·61) could be an ester, although this is uncertain as the RPA value lies on the border between ester and aldehyde. Peak 11a (5·00) would most probably be an alcohol, peak 17 (2·68) an aldehyde, peak 19 (1·64) an ester (again uncertain), peak 19a (1·22) an ester and peak 24 (2·84) an aldehyde. This information could be used to indicate which

pure compounds should be tested to find retention times which are in agreement with the unknown peaks of the apple juice.

Influence of salt addition when comparing gas chromatographic headspace analysis and sensory evaluation

In the previous sections, the influence that the addition of salt to apple juice has on the amount of various volatile compounds found in the headspace by gas chromatographic analysis has been discussed. In particular, it has been noted that, with salt addition, alcohols are more distributed in the headspace than are aldehydes or esters. This alteration of the composition of the headspace can have very important consequences when attempting to relate volatile composition, measured by headspace chromatograms, with sensory evaluations conducted on apple juice samples to which salt has not been added. To examine the effect of these changes, sensory aroma evaluations were conducted on the apple juice samples used for the gas chromatographic headspace analyses reported in Table 1.

Before discussing the results of the correlation study, it should be noted that, since the aroma sensory tests were conducted at room temperature, we had, without considering it at the time, actually had a potential headspace enrichment effect due to the temperature difference between the GC analysis (40 °C) and the sensory analysis (20 °C). A small, model solution experiment was therefore conducted after completion of the main study, demonstrating that, at 20 °C, salt had a greater effect on headspace enrichment (RPA values were 2–3·5 times higher) than at 40 °C. The *differences* of RPA values for esters, aldehydes and alcohols, while lower at 20 °C than 40 °C, were still sizeable. From these results it was judged that the discussion presented below, relating sensory and headspace analyses for the apple juice samples, is valid, even though the analyses were conducted at different temperatures.

Results of the sensory evaluations are given in Table 3 and Fig. 2. Table 3 also includes total peak areas for the esters, aldehydes and alcohols alone, the total peak area for all components, and the percentage distribution of the headspace amongst the three major classes—esters, aldehydes and alcohols. From Fig. 2 it can be seen that, as salt is added to the apple juice, aroma intensity and off-aroma both increase, while fruit aroma is essentially constant. Results of analysis of variance tests for the sensory evaluation data, which are given in Table 3, further support these

TABLE 3
Sensory Aroma Scores, Summed GC Peak Areas and Percentage Distribution of Peak Areas for Apple Juice at Various Levels of Added NaCl

	Grams of salt per 100 ml of juice			
	0	*12·5*	*25·0*	*37·5*
Aroma evaluation (point scale 0–10)				
Intensity*	4·80 (*ab*)	6·30 (*bc*)	7·25 (*c*)	9·0 (*d*)
Fruit aroma	4·60 (*e*)	4·50 (*e*)	4·20 (*e*)	3·80 (*e*)
Off-aroma	1·10 (*fg*)	2·30 (*gh*)	3·25 (*h*)	3·75 (*h*)
GC peak area				
Sum of esters	1 865	2 031	2 078	2 536
Sum of aldehydes	403	505	682	1 033
Sum of alcohols	789	1 323	2 505	4 295
Sum of all components	3 057	3 859	5 265	7 864
Per cent distribution				
Esters	61·0	52·6	39·5	32·3
Aldehydes	13·2	13·1	13·0	13·1
Alcohols	25·8	34·3	47·6	54·6
All components	100	100	100	100

* Samples with the same letter in parentheses are not statistically different at the 95% level.

conclusions. It can be seen that no fruit aroma scores were significantly different (95% level), whilst significant differences were found for both aroma intensity and off-aroma.

It can thus be seen that addition of salt has significant consequences on the sensory evaluation of apple juice based on aroma variations. The increase in aroma intensity can be related to the increase in total peak area or peak area of each class of compound. As has been mentioned earlier, the increase in peak area for the alcohols is much larger than for either the esters or aldehydes. With no salt added, esters constitute roughly 61% of the total headspace composition based on peak area, while alcohols are only 26%. Aldehydes comprise the remaining 13% and their percentage remains essentially unaltered with salt addition. With the addition of salt, the percentage of the headspace coming from esters falls drastically, to about 30% in the salt-saturated apple juice. In such samples, alcohols comprise 55% of the headspace. The results of these changes are a headspace with a very different composition from samples having no salt addition.

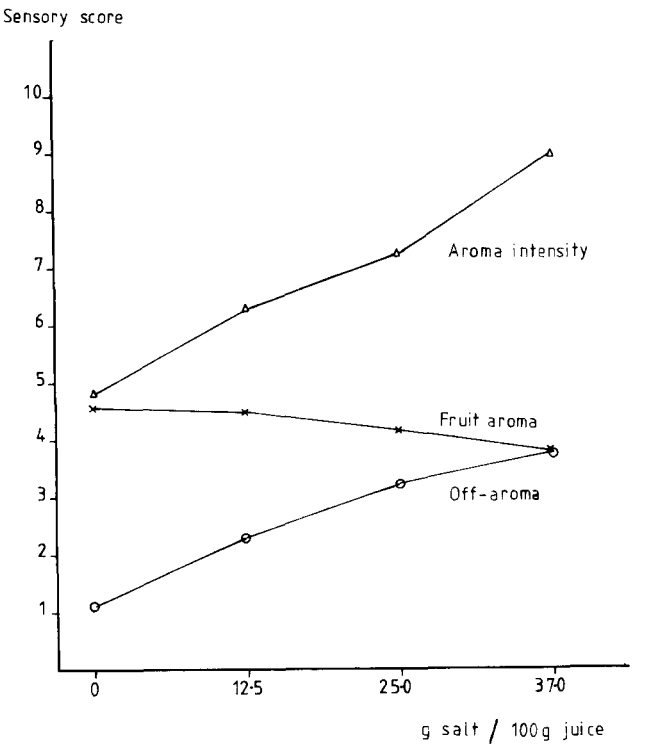

Fig. 2. Sensory aroma scores for apple juice samples containing various levels of added NaCl.

These changes in percentage distribution of the headspace appear to have little effect on the scores for the fruit aroma of the sample whereas they seem to have a significant effect on the off-aroma. It is tempting to relate the changes in the per cent distribution of the headspace components with the off-aroma values which have been determined by sensory evaluation. It appears that the increased percentage of alcohols could be correlated with the increased presence of off-aroma in the samples ($r = 0{\cdot}9860$). Similar results have been found by Poll (1983) for changes in apple juice aroma following 1 year's storage at temperatures of 3 to 30 °C. With increasing storage temperature, fruit aroma decreased and an off-aroma, characterized as cooked-aroma, increased. Headspace GC analyses of these samples showed that while all volatile components decreased, alcohols decreased at a slower rate than esters or aldehydes, resulting in the alcohol percentage in the headspace being greater with increasing storage temperature.

From data in Table 3, it can be noted that the sum of esters and aldehydes, expressed as a percentage of the total headspace, followed a similar trend to fruit aroma score, expressed as a percentage of the sum of fruit aroma and off-aroma scores, as salt was added to the sample.

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