Extraction of Volatile Compounds from Green Peas (*Pisum sativum*)

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The volatile fractions extracted from unblanched green peas by three distillation techniques and by vacuum sublimation were of similar qualitative but markedly different quantitative composition. With blanched peas the extracts provided by all the methods examined were identical. The use of distillation methods to extract biologically active materials involves a serious risk of compositional changes in the volatile fraction during extraction. Vacuum sublimation, conducted under conditions which provide maximum practical protection against enzymic, microbial, and thermal changes, is recommended as a reference method for assessing the authenticity of extracts derived by distillation methods.

Green peas intended for commercial processing are harvested mechanically. During the vining operation they are physically damaged. During the subsequent delay, prior to blanching, such peas develop off-flavor, the intensity of which is determined by the duration of the delay and the temperature of the peas. It is most likely that the off-flavor results from abnormal enzyme activity in the damaged peas. However, since the peas are liable to microbial contamination during vining, it is possible that microbial spoilage is also involved.

Shipton and Last (1968) showed that the development of off-flavor, measured organoleptically, is accompanied by an increase in volatile content. This suggested that, in view of the very low volatile content of peas, it would be advantageous, in the investigation of the chemical composition of the volatiles in off-flavored peas, to use peas which had been held at ambient temperature for 6 to 8 hours after vining. In preliminary studies the volatiles were extracted by distilling a purée of such peas at 10° C. and 8 to 10 torr. It was recognized that these conditions would not preclude enzyme or microbial activity, but it was considered that any such activity was unlikely to differ qualitatively from that occurring during the delay period, and, therefore, would not vitiate a qualitative analysis of the volatiles.

Despite the enhanced volatile content of delayed vined peas, the distillates derived from them had a very low level of nonaqueous constituents, and it was necessary to devise techniques for their concentration (Shipton and Whitfield, 1966; Whitfield and Shipton, 1966). The concentrates obtained have permitted the identification of more than 50 constituents (Murray *et al.*, 1968a, 1968b) and elucidation of others is continuing.

These techniques provided data about the volatile composition of off-flavored peas, but, to determine the components responsible for the off-flavor, it was necessary to compare, qualitatively and quantitatively, the volatile composition of off-flavored peas with that of normal peas. To establish the latter it was essential that the conditions of extraction and concentration would prevent any enzymic, microbial, or thermal changes in the volatiles. Hence, a study of possible methods was undertaken.

The techniques which have been used for preparing food volatile concentrates, apart from a few based on direct solvent extraction (Andersson and von Sydow, 1964; Heinz *et al.*, 1966) utilize some form of distillation. They vary widely in the degree of assurance they provide against compositional changes. Pyne and Wick (1965) used low-temperature vacuum stripping to recover the volatiles from carefully prepared purées and claimed that this minimized the possibility of artifact formation. However, since significant enzyme activity is likely at temperatures above the freezing point, it is clear that none of these distillation techniques completely precludes modification, during extraction, of the volatile fraction of biologically active foods.

The method which appeared most likely to avoid these difficulties was one in which the volatiles are sublimed from the frozen food. This method involves trapping the vapors emanated during freeze-drying. It has the obvious disadvantages of being time-consuming and yielding extracts in very dilute aqueous solution. However, the latter, being free from enzymes and microorganisms, can be concentrated under less stringent conditions. Further problems, common to all volatile extraction procedures, are that quantitative recovery cannot be assumed, and that, due to their diverse vapor pressures, the volatiles may not be removed in proportion to their concentration in the food. Despite these deficiencies, vacuum sublimation appeared to be, at least for biologically active foods, the best means of obtaining authentic extracts, thus constituting a reference method against which the acceptability of other procedures might be assessed.

This paper presents the results of a study of the extraction of the volatiles from green peas by low-temperature vacuum distillation, with and without salt saturation of the distilland, and by low-temperature vacuum sublimation.

MATERIALS AND METHODS

Green peas (*Pisum sativum* var. Edgell Freezer) grown at Richmond, N.S.W., were harvested, vined, washed, and held for 4 hours at a mean ambient temperature of 26° C. Half of the crop was frozen unblanched and the other half after blanching in boiling water for 90 seconds, water-cooling, and draining. All material was frozen by immersion in liquid nitrogen. This method of freezing caused the peas skins to split with, in most cases, separation of the cotyledons. The rupture of the skin, which constitutes an effective barrier to water vapor transmission, greatly increased the drying rate in subsequent treatments involving vacuum sublimation.

The frozen peas were packed in 0.004-inch thick polyethylene bags and stored for about 6 months at −30° C.

The methods used for extracting and concentrating the volatiles from these peas are described under Experimental. All comparisons were carried out in duplicate.

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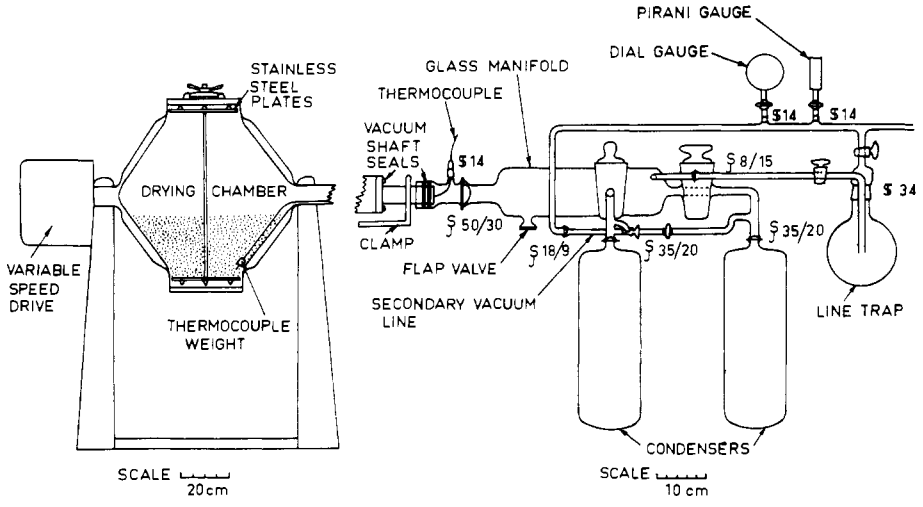

Figure 1. Vacuum sublimation apparatus

The terms extract and essence are used in this paper with the following meanings.

Extract. The volatile fraction derived by low-temperature vacuum distillation, through two in-series cold (0° C.) reflux condensers, of a puréed food or of a dilute aqueous solution.

Essence. The volatile fraction resulting from the concentration of an extract.

The essences were examined by gas chromatography using a stainless steel column (10-feet × 1/4-inch O.D.) packed with 60- to 70-mesh nonacid washed Chromosorb G coated with 5% Carbowax 1540. The oven temperature was programmed from 60° to 150° C. at 5° per minute and then held at 150° C. for an additional 33 minutes. A hydrogen flame ionization detector and nitrogen carrier gas were used.

Prior to gas chromatography the volumes of the essences were adjusted with glass distilled water so that they were appropriately related to the weight of peas from which they were derived. This permitted samples of equal volume to be injected onto the column to provide chromatograms which, quantitatively, could be compared directly. The chromatograms revealed 32 common peaks of which seven were selected for quantitative assessment. Their concentrations, relative to those in the essence obtained from the vacuum sublimation treatment, were derived by comparison of peak areas as measured by disk integration. The absolute concentrations of the seven components in the vacuum sublimation essence were determined with the assumption that each peak comprised only its major constituent, as previously identified by mass spectrometry (Murray *et al.*, 1968a). The gas chromatograph was calibrated with a series of standard aqueous ethanolic solutions of these compounds. By comparison with the peak areas obtained, the absolute concentrations of the seven compounds were established. These data were used to calculate the amount of each of these compounds which was recovered from green peas by the vacuum sublimation method.

EXPERIMENTAL

Extraction of Volatile Compounds from Green Peas by Vacuum Distillation. The apparatus comprised a thermostated water-bath, which housed a 10-liter flask, fitted with a mechanical stirrer, and connected, through two in-series low-temperature (0° C.) double surface reflux condensers (Davies, S 19), to a mechanical vacuum pump. The vacuum line between the condensers and pump was fitted with a sample-trap and a back-trap, both cooled in liquid nitrogen

Three distillation techniques were examined.

Distillation Method A. Frozen (−30° C.) peas (6 kg.) were rapidly minced into the 10-liter flask. After connecting the flask, the apparatus was carefully evacuated with continuous stirring of the purée. When the pressure dropped to 6 torr, the vacuum pump was disconnected and the water-bath temperature adjusted to 23.5° C. Under these conditions, distillation was established at a rate which produced partial flooding of the lower condenser with only slight condensation visible in the upper one. The distillation was continued for 6 hours, during which time the pea purée temperature rose gradually from −30° to +15° C. The extract (about 30 ml.) which had collected in the sample-trap was thawed, saturated with sodium chloride, and stored at 0° C. When derived from peas with a high content of hexan-1-ol, the thawed extract showed visible separation of oil globules.

Distillation Method B. The procedure was similar to that of method A except that sodium chloride (2.1 kg.) was mixed with the peas immediately prior to mincing.

Distillation Method C. The initial procedure accorded with that of method A except that the reflux condensers were not used. The distillate (about 1 liter) was saturated with salt, placed in a 2-liter flask, and distilled as described in method A until 30 ml. of extract were collected. A preliminary comparison revealed that this technique yielded extracts of identical composition to those provided by method A. Since the latter involved only a single-step distillation, method C was not included in subsequent investigations.

Extraction of Volatile Compounds from Green Peas by Vacuum Sublimation. The vacuum sublimation equipment is depicted in Figures 1 and 2. To permit operation at submicron pressures and to eliminate contamination by atmospheric leakage, the original vacuum shaft seal was replaced by a set of synthetic rubber "O" rings, which were lightly lubricated with Apiezon M vacuum grease. The condensers connected to the glass manifold were cooled by liquid nitrogen contained in 10-liter vacuum flasks. The level of the liquid nitrogen was maintained by differential level controllers. During preliminary runs it was observed that a significant temperature gradient existed across the pea mass in the drying chamber. An unweighted thermocouple tended to float on the surface of the pea mass where the temperature was markedly lower than at the wall of the drier. Reliance on the temperature indicated by the thermocouple

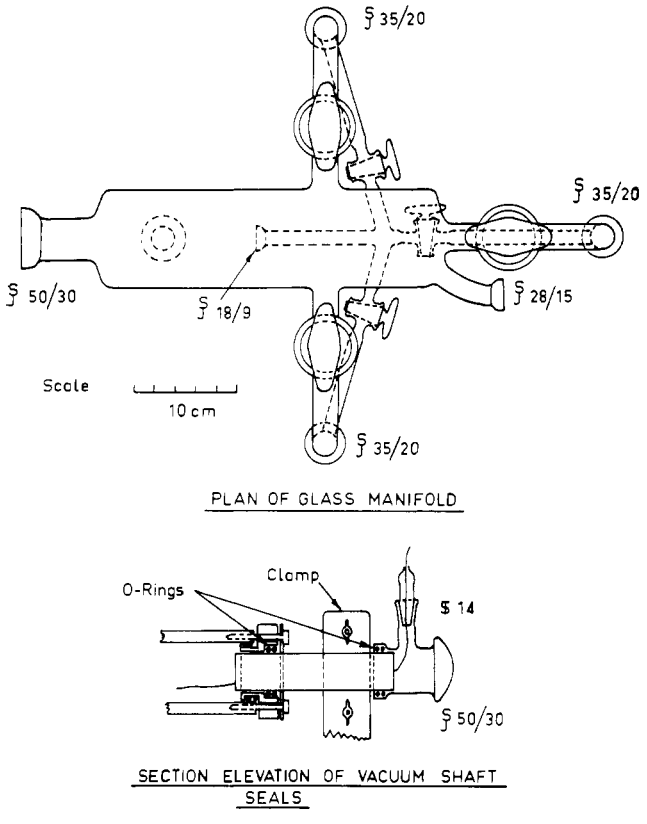

Figure 2. Details of glass manifold and vacuum shaft seal sections of vacuum sublimation apparatus

under these conditions involved a serious risk of thawing peas near the wall. By weighting the thermocouple the measuring junction was kept adjacent to the wall and satisfactory operation was achieved.

The drying chamber was rotated at 2 r.p.m. At higher speeds, the peas, when partially dry, tended to disintegrate and cause dust to pass into the condensers.

The secondary vacuum line enabled each of the condensers to be evacuated while isolated from the manifold, thus permitting the condensers to be changed without interrupting the sublimation.

The unjacketed ends of the drying chamber transferred heat significantly faster than the jacketed wall areas. In preliminary runs thawing and sticking of the peas occurred at the end walls. The stainless steel plates (Figure 1) were inserted to prevent the peas from contacting the unjacketed end walls.

The manifold incorporated a simple flap valve which opened if the internal pressure exceeded atmospheric pressure. This prevented any hazardous pressure development if all the liquid nitrogen used for preliminary cooling had not evaporated before the drying chamber was sealed.

The equipment was operated as follows. The entire system was evacuated to 10^{-3} torr, with only the back-trap cooled, and allowed to stand overnight. This served to desorb gases from the inner walls. The system was then pumped to $<10^{-3}$ torr, and the condensers and traps isolated by closing the appropriate stopcocks. The vacuum was released in the manifold and drying chamber. The inner walls of the latter were cooled with about 10 liters of liquid nitrogen. When the liquid nitrogen had almost wholly evaporated, the frozen peas (22.5 kg. precooled to $-40°$ C.) were placed in the chamber, which was then sealed. The pressure was reduced to 20 torr with only the back-trap operating. Due to the low temperature of the peas, no detectable loss of volatiles occurred above

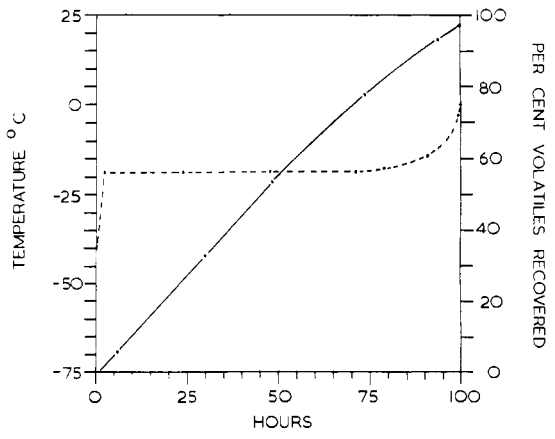

Figure 3. Temperature of peas and progressive volume of volatiles collected during a typical vacuum sublimation

·——· % volatiles; ····· temperature

this pressure. At 20 torr the line-trap was cooled with liquid nitrogen, the drying chamber rotation started, and pumping was then continued until all noncondensable gases from the peas had been removed. It was necessary to effect this degassing before opening the condensers as their efficiency was seriously impaired if they contained significant volumes of noncondensable gases. The condensers, cooled in liquid nitrogen, were now opened to the manifold. Once the pressure dropped to 10^{-3} torr, pumping was discontinued, and thermostatically controlled radiant heating of the outer wall of the drying chamber jacket was used to maintain the temperature of the peas at not less than $-18°$ C.

Sublimation was conducted for 100 hours, the condensers being changed as necessary. With this procedure 97% (17.4 liters) of the water content of the peas sublimed to the condensers and the temperature of the peas did not exceed 0° C. (Figure 3). The sublimate, after thawing, was saturated with sodium chloride and stored at 0° C.

The nonaqueous volatile constituents of the sublimate were concentrated using the technique described for distillation method A. The extract (about 60 ml. from 17.4 liters of sublimate) was saturated with sodium chloride and stored at 0° C.

Concentration of Extracts. All the extracts were concentrated using the equipment described by Shipton and Whitfield (1966) modified by omitting the U-tube separator.

The cold (0° C.) sodium chloride saturated extract (30 or 60 ml.) was distilled from a 100-ml. flask. The solution was stirred, the condensers cooled to 0° C., the receiver cooled in liquid nitrogen, and the water-bath adjusted to 11.5° C. The system was evacuated to 20 torr. These conditions were maintained for 10 minutes and the bath temperature was then increased by 2° increments to 23.5° C. The intervals between increments were regulated to maintain partial flooding of the lower condenser. When the bath temperature reached 23.5° C., the same conditions of reflux were subsequently held by reducing the pressure in steps of 1 torr until it reached 6 torr, when pumping was stopped. After 6 hours' distillation the contents of the receiver were removed (essence fraction 1). A further 2 hours' distillation yielded essence fraction 2. The volumes of fractions 1 are given in Table I. The greater volume of essence obtained from unblanched peas by distillation method A is mainly due to its much higher ethanol content (Table II). This increased amount of ethanol was generated during the distillation process in which no effective procedures were adopted to restrict enzyme activity.

The extracts, before salt saturation, and the two essence

Table I. Volume of Essence Fraction 1

Process	Extraction Method	Volume, Ml.
Unblanched	Distillation method A	12.0
	Distillation method B	4.3
	Vacuum sublimation	4.2[a]
Blanched	Distillation method A	4.5
	Distillation method B	4.2
	Vacuum sublimation	4.6[a]

[a] Calculated volumes for 6 kg. of peas.

Table II. Concentrations (Relative to Those in the Vacuum Sublimation Essence of Unblanched Peas) of Constituents in Distillation Method Essences

Peak No.	Blanched[a] Chromatogram D[b]	Unblanched: Distilled unsalted purée chromatogram A[b]	Unblanched: Distilled salted purée chromatogram B[b]	Identity of Major Component of Peak
1	P	VMS	S	...
2	S	P	P	Ethanal
2a	P	A	A	...
3	P	S	P	...
4	W	P	W	...
5	P	P	P	Methanol
6	P	S	P	Ethanol
7	P	S	P	Propan-1-ol
8	W	P	W	...
9	S	P	S	Butan-1-ol
10	W	MS	S	Pent-3-en-1-ol
10a	W	A	A	...
11	W	W	W	2-Methylbutan-1-ol + 3-Methylbutan-1-ol
11a	A	A	P	...
12	S	MS	S	Pentan-1-ol
12a	W	P	P	...
13	P	MS	MS	Pent-2-en-1-ol
14	MW	VMS	P	Hexan-1-ol
14a	P	A	P	...
15	MW	MS	P	Hex-3-en-1-ol
16	A	MS	W	...
16a	A	A	P	...
17	S	MS	P	Oct-3-en-1-ol
18	P	MS	P	Heptan-1-ol
19	P	P	S	Hept-3-en-1-ol
20	W	S	S	Hept-2-en-1-ol
20a	P	A	P	...
21	W	MS	P	Octan-1-ol
22	MW	P	W	...
23	S	MS	P	Oct-2-en-1-ol
24	P	S	P	...
25	W	S	P	Nonan-1-ol
26	P	MS	W	Non-3-en-1-ol
27	A	P	P	...
28	P	P	P	...
29	P	P	P	Naphthalene
30	P	W	P	Benzthiophene
31	P	W	P	2-Methylnaphthalene
32	P	P	P	1-Methylnaphthalene

[a] The blanched essence chromatograms also possessed peak numbers 8a, 13a, 13b, and 25a. [b] P = similar; A = absent; W = weaker; MW = much weaker; S = stronger; MS = much stronger; VMS = very much stronger.

fractions from each, were examined by gas chromatography. Essence fraction 2, in all cases, was shown to be principally water with a trace of ethanol as the only detectable organic constituent. Each essence fraction 1 was qualitatively identical with its parent extract.

RESULTS AND DISCUSSION

When applied to blanched (enzyme inactivated) peas each of the extraction techniques yielded gas chromatographically identical essences (Figure 4D). While this does not imply that the methods provided quantitative recovery of all, or any, of the volatile constituents, it does indicate that their extraction efficiency was similar for all components and thus, for biologically inactive material, each provided equally valid data. Furthermore, it suggests that if these techniques, when applied to biologically active material—e.g., unblanched peas—yield dissimilar essences, the quantitative or qualitative variations in the latter would reflect a difference in the extent of biological activity occurring during isolation rather than a difference in the recovery efficiency of the methods.

Chromatograms A, B, and C (Figure 4) show the composition of essences obtained from unblanched peas by distillation methods A and B and by vacuum sublimation, respectively. Clearly these essences were qualitatively very similar, some 32 peaks being common to each. Peaks 10a, 11a, 14a, 16a, and 20a in chromatogram C are absent from chromatogram A and peak 10a is also missing from chromatogram B. However, as peak 10 is significantly larger in A and B than in C, it is possible that peaks 10a and 11a were not resolved. In contrast to this qualitative similarity, there were striking quantitative disparities between the essences. That obtained by distillation of the unsalted purée (chromatogram A) contained 17 components in appreciably higher concentrations than that prepared by vacuum sublimation (chromatogram C), while only seven components of the essence from the distilled salted purée (chromatogram B) showed a similar disparity. The relative concentrations and, where known, the identities of the constituents of these essences and also of blanched pea essences are given in Table II. The quantitative differences between the unblanched pea essences must have resulted from synthesis or degradation of constituents during extraction.

Concentrations, relative to those in the vacuum sublimation essence from unblanched peas, for seven of the 32 common peaks are given in Table III. With few exceptions, the essence from vacuum sublimation had the lowest, and that from distillation of unblanched unsalted pea purée the highest, concentration of each component. The data in Table III show that the addition of sodium chloride to the frozen peas prior to mincing significantly inhibited biological activity during the subsequent distillation. However, the degree to which their production was inhibited was not constant for all components. It appears to have been complete for peak 14 (hexan-1-ol) but negligible for the combined peaks 9, 10 (butan-1-ol, pent-3-en-1-ol), and peak 13 (pent-2-en-1-ol).

These results demonstrate the risk of compositional changes when distillation techniques are used to extract volatiles from biologically active materials. They show that vacuum sublimation is preferable although, even here, the possibility of some biological activity cannot be denied since no absolute reference standard is available.

The temperature of the peas and the progressive volume of volatiles, including water, recovered during a typical sublimation are shown in Figure 3. Since the volatiles are composed almost entirely of water the recovery data do not infer any specific efficiency for the recovery of the nonaqueous components.

Since sublimation was effected at low temperature, low water activity, and in the absence of oxygen, it is unlikely that artifact formation by enzymic, microbial, or thermal means would have occurred. Thus the qualitative volatile composition of a food is more truly reflected in essences derived from vacuum sublimates than in those prepared by any other method. Hence, this procedure is proposed as suitable for preparing reference essences against which may be assessed

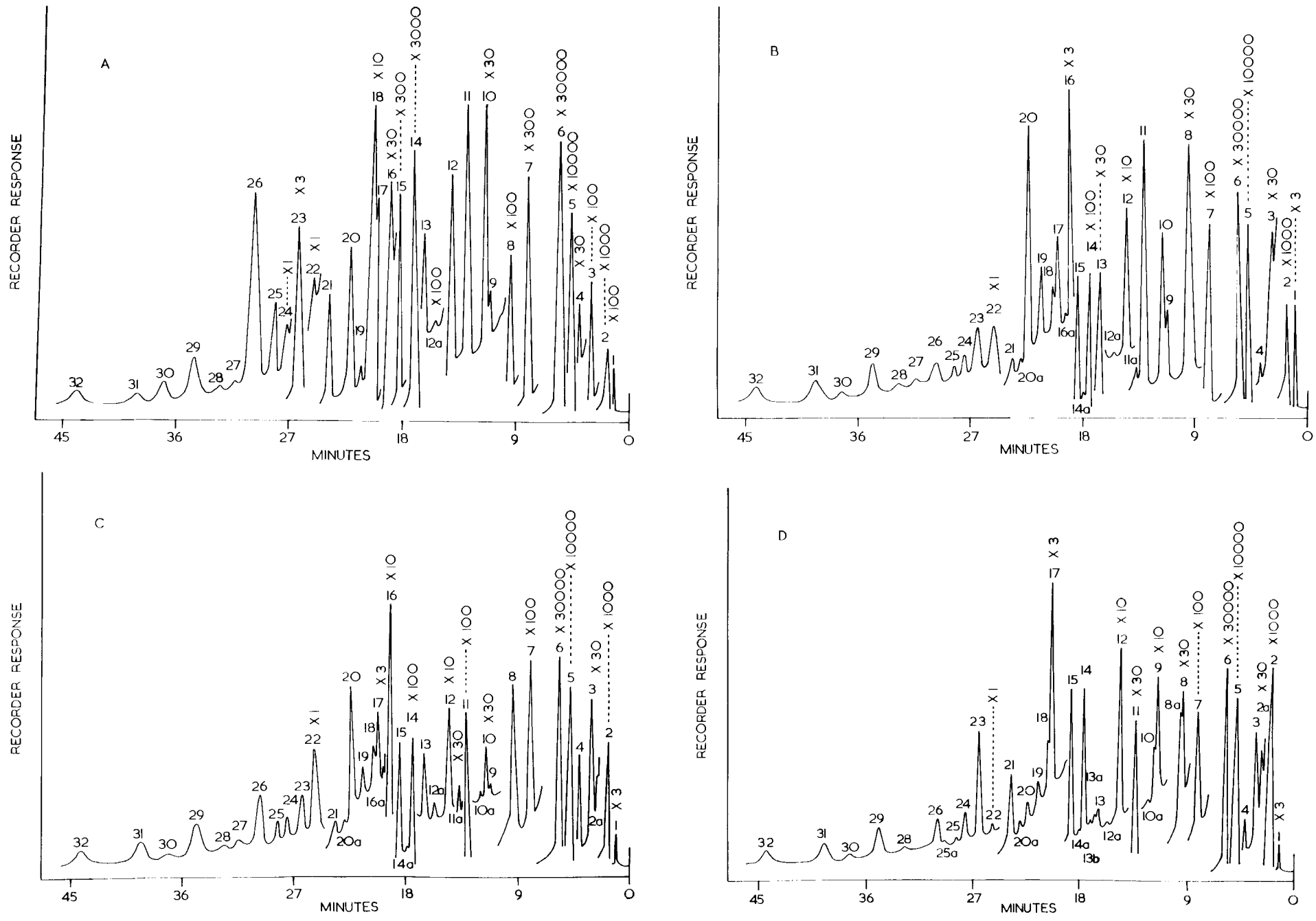

Figure 4. Chromatograms of essences prepared by

A. Distillation of unblanched unsalted pea purée B. Distillation of unblanched salted pea purée C. Vacuum sublimation of unblanched peas D. Extraction of blanched peas by any of the above methods

Table III. Relative Concentrations of Seven Selected Components of Pea Essences as Influenced by Method of Extraction

Process	Extraction Method	Peak No.[a] 6	9–10	11	12	13	14	15
Unblanched	Vacuum sublimation	1 (400)	1 (0.014)	1 (0.16)	1 (0.01)	1 (0.005)	1 (0.12)	1 (0.14)
	Distillation method A	2.5	8	0.7	13	11	100	6
	Distillation method B	1	6	0.7	2.5	10	1	1
Blanched	Vacuum sublimation Distillation method A Distillation method B	1	1	0.3	1.5	1	0.1	<0.1

[a] Figures in parentheses are calculated concentration (p.p.m.) of these components in unblanched peas.

the qualitative authenticity of essences derived by other techniques.

As shown in Table III, the vacuum sublimation essence contained much smaller amounts of many of the components with boiling points higher than that of ethanol than did those from the distillation methods. In the vacuum sublimation essence these components, of which hexan-1-ol comprised 24%, collectively represented a recovery of approximately 0.5 p.p.m. from the raw material. Despite its low concentration this higher boiling fraction is of major organoleptic interest.

The distillation of unblanched unsalted purée yielded an essence, of which the higher boiling fraction, containing 90% hexan-1-ol, represented a recovery of 13.5 p.p.m. from the raw material. Although this method permits biological activity during distillation, it has proved most valuable in these investigations since it provided high yields of essence which differed only slightly in qualitative composition from the vacuum sublimation essence. The hexan-1-ol content of the extract obtained using distillation method A sufficed to cause it, during concentration of the extract by the Shipton and Whitfield (1966) technique, to separate as an oil and subsequently to act as a solvent for the components with boiling points above that of ethanol. This obviated the need for solvent extraction. The relative abundance of the essence greatly facilitated the identification of the higher boiling volatile constituents of unblanched peas (Murray *et al.*, 1968a, 1968b). This knowledge was most useful in the subsequent identification of the same components in the greatly reduced yields of essence obtained from blanched peas or by vacuum sublimation.

Thus, although distillation methods may have defects they can yield valuable data when employed with a full awareness of their deficiencies. Their limitations for any particular raw material must be assessed. This may be done by using vacuum sublimation as a reference standard. Only by the use of such a precaution can the validity of data based on distillation methods be established.

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Received for review October 21, 1968. Accepted April 23, 1969