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(Publikatie uitsluitend met toestemming van de directeur)

RAPPORT NO. 2048

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SELECTION OF TOMATOES BASED ON COLOUR AND ETHYLENE PRODUCTION FOR PREPARATION OF SAMPLES TO BE USED IN SUBSEQUENT STUDIES OF ENZYME ACTIVITIES AND FIRMNESS

Uitgebracht aan de directeur van het Sprenger Instituut
Proj.no. 102

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Summary

For preparation of a homogeneous sample of tomato at pre-climacterium, climacterium and post-climacterium stages, selection based on clour should be complemented with a second selection based on ethylene concentration (or production).

Introduction

We started with a project about the (bio)chemical/physical characterization of tomatoes. In this project a hybride between 'rin' and Moneymaker was prepared.

One of the objectives is to characterize the keepability through the parameters of the enzyme activities of superoxide dismutase (SOD) and peroxidase (PO), and also through firmness.

Based on the work of Frenkel and Garrison (1976), Brenman and Frenkel (1977) and Chalutz et al (1977) oxygen is necessary for the start in the biogenesis of ethylene.

Yang (1975) also demonstrated that oxygen was essential for conversion of methionine to ethylene by apple tissue.

Lieberman and Mapson (1964) showed that methionine is the precursor of ethylene in plants. Methionine was rapidly converted into ethylene in a model system consisting of Cu^{2+} and ascorbic acid. In this conversion methional (3-methylthiopropionaldehyde) appears to be an intermediate.

Lichtfield and Wells (1978), produced ethylene from methional in the presence of hydrogen peroxide (Fig. 1).

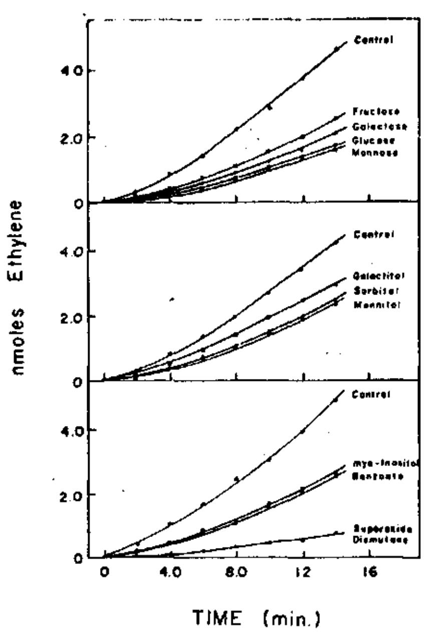

FIG. 1. Inhibition of hydroxyl radical-dependent degradation of methional. All carbohydrate and polyol levels were 40 mM. Benzoate and superoxide dismutase levels were 5 mM and 0.2 mg/ml, respectively.

(from Lichtfield and Wells (loc. cit.))

The non-enzymatic system contained 1.0 mM H_2O_2 and 1.0 mM methional. Lichtfield and Wells,(loc. cit.) postulate the following four reactions in which iron, as a contaminant of phosphate buffer, is oxidized by H_2O_2 and reduced by superoxide anion:

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH\cdot + Fe^{3+}, \quad [1]$$

$$H_2O_2 + OH \rightarrow OH^- + O_2^{\cdot -} + 2H^+, \quad [2]$$

$$O_2^{\cdot -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}, \quad [3]$$

$$CH_3SCH_2CH_2CHO + OH \rightarrow \tfrac{1}{2}(CH_3S)_2 + \underbrace{CH_2CH_2}_{\text{ethylene}} + HCOOH. \quad [4]$$

The above reactions were also described with more details by Beauchamp and Fridovich (1970).

How SOD inhibited the production of ethylene was not explained by Lichtfield and Wells (loc.cit.), probably because their scope was the scavaging of free radicals by galactose in respect of polymorphonuclear leucocytes and ethylene production was not their principal objective.
Our explanation for the strong inhibition of ethylene production by SOD could be as follows.
Reaction [2] is shifted to the right by depletion of O_2^- [a]. So the steady state conc. of OH· is lowered.
Recently Mattoo and Lieberman (1977) demonstrated with cultivated apple cells that the biogenesis of ethylene starts only when apple spheroplasts grew cell walls. The machinery for biosynthesis of ethylene would be in the cell wall - cell membrane complex.
We think that SOD is in some way associated with permeability or integrity of membranes and as long as SOD scanvanges O_2^- less injury of membrane occurs.
Our hypothesis is that an organ (tomato) that has more SOD activity will have more keepability. In this manner the keepability could be seen as a potential concept (Rudolphij et al).
It is known that 'rin' tomato is a mutant that does not ripe whereas Moneymaker is a classical with climacterium. The hybride would show indirectly the genetical influence of both parents. Genetical influence as it is regulated by genes would support the concept of potentiality as basic idea of keepability..

(a) This explanation holds only in vitro! because it supposes an excess of H_2O_2, necessary in reaction [2]. In vivo, H_2O_2 is quite small due to catalase and peroxidase

In concreto, our hypothesis is that SOD activity of rin will be higher than of hybride and SOD of hybride higher than Moneymaker.
Before preparing acetone powders as source of enzymological studies we found that it was essential to apply a double selection for preparation of homogeneous samples. The first selection was via colour (stages as mentioned by Stenvers, 1976) and afterwards by ethylene concentration. Ethylene concentration was converted to production by calculation, once the samples were already selected on basis of concentration. The ethylene production permitted to define the climacteric stage: preclimacteric, climactetic, post climacteric.
We shall confine in this report to the estimation of ethylene production. Enzymological studies are being made and they will be informed - as well as firmness - in a separate manuscript.

Materials and methods

'Rin', Moneymaker (MM) and hybride (F_1) were cultivated by dr. Hogenboom (IVT, Wageningen).
Tomatoes of F_1 and MM were picked at the assumed stages of unripe (pre-climacterium), ripe (climacterium) and overripe (post-climacterium) as judged by colour (Table 3). 'Rin' tomatoes were picked when green and when yellowish. After picking in the early morning, the tomatoes were left at 20^{o}C. After about two hours each tomato was submitted separately to the estimation of ethylene production, that was also carried out at 20^{o}C.

Estimation of ethylene production

Each tomato was placed in a container of 0.664 L $\pm$ 0.002 L (coefficient of variability 0.3%, n = 6 containers sampled at random that could be closed hermetically (see photo of Fig. 2)).
After some preliminary experiments it was decided that incubation of the tomato in the said container would be for 40 minutes. This period of time was necessary to build up a sufficient ethylene concentration in the atmosphere to be measured by gas liquid chromatography.(Gorin and Honkoop, 1978) and safe enough for avoiding anaerobiosis or induction of ripening by accumulation of too much ethylene.
As illustration we provide in Table 1 the data pertaining to MM, green. At that moment, tomatoes with similar concentration (ppm) were selected for preparing a homogeneous sample. Of course the concentration is not a defined fruit parameter, as is the production, but still dependent on the fruit volume, wich varies considerably. However concentration was taken for fast selection of fruits.

Calculation of ethylene production

$$P = \frac{\text{Net Vol. } \times \Delta C \times 1.5}{100}$$, where

P = production of ethylene,
Net Vol. = Volume of container (0.664 L) minus vol. of tomato.
Volume of tomato was calculated as follows: weight of tomato (g) divided specific gravity. The specific gravity used was 0.98 that is an average of two extreme values (0.957 and 1.006) given by Stenvers (loc.cit.).
ΔC is the accumulated concentration of ethylene (ppm or $\mu L \cdot L^{-1}$) in 40 minutes.
Factor 1.5 is to convert value of 40 minutes into 60 minutes (one hour).
Denominator 100 is to convert per 100 g tomato into g tomato. We refer ethylene production per g tomato.
Production ($nL.h^{-1} \cdot g^{-1}$) was used in order to compare data of MM with graph given by Knegt et al (1975) (see Fig. 3).
Table 2 records - as illustration - the data of the tomatoes of Table 1 selected for preparation of acetone powders. The same procedure was used for all kinds of samples mentioned in Table 3.
The following example is given for showing as calculation of ethylene concentration (tomato Nr. 1 of Table 2) was done.

$$P = \frac{0.598 \text{ L} \times 19 \cdot 10^{-3} \ \mu L \cdot L^{-1} \times 1.5}{100} = 0.170 \times 10^{-3} \ \mu L \cdot h^{-1} \cdot g^{-1} \text{ or}$$

taking out 10^{-3} (nL) it is 0.170 $nL \cdot h^{-1} \cdot g^{-1}$.
0.598 L is Net Volume that originates as follows. Vol. of container 664 ml minus vol. of tomato 66 mL = 598 mL of 0.598 L.
Vol. of tomato (66 mL) was obtained by dividing weight of tomato (64.315 g) by specific gravity 0.98 = 66 mL.

Results and discussion

Table 3 records the data of several samples of tomato and the respective ethylene production. It is clear that colour alone is not sufficient to select organs for preparation of a homogeneous sample.
In new experiments, it would be better to reach a maximum coefficient of variation of ± 20%.
If we compare ethylene production of MM with the graph of Knegt et al (1975) we deduce that we selected all right: pre-climacterium, climacterium and post-climacterium.
We prefer to speak from now on of samples pertaining to pre-climacterium, climacterium and post-climacterium stages and not to use the words unripe,

ripe and overripe. These last three words are better used in eating-quality sense.

Unfortunately we have not a typical climacterium curve for F_1, as F_1 is new in the Netherlands and perhaps in the world.

However, table 3 clearly indicates that the ethylene production of F_1 is lower than of MM and higher than rin. Lower ethylene production would indicate, at first sight, higher keepability. However this statement can be affirmed once the results of firmness have been published. In any case, the influence of 'rin' on the hybride is clearly seen.

Acetone powders of all samples mentioned in Table 3 have been prepared in the same day that measurements of ethylene concentration were done. We already detected SOD and PO activities.

Table 4 records that data of tomatoes given for Hunter-colour estimations and firmness. Both data will also be published elsewhere.

The advantage of acetone powders is that phenolic compounds that denature enzymes have been removed. The disadvantage is that the natural membranes have been damaged. Therefore we should consider the enzyme activities determined via acetone powders as a first approach and later to pass to work with disks (slices), as it is done by Feys, Leuven. In disks the membranes and systems of most cells of the tissue are intact and they approach more the reality of the whole organ.

Perspective and recommendations

Perspective

Scavaging of free radical (superoxide as well as other free radicals) brings a new light on the protective actions of substances against the start of ethylene production in horticultural produce.

Recommendations

1. The trial should be repeated with a new harvest (1978), otherwise results obtained with only one harvest are a coincidence.
2. An exact repetition of the trial, would implicate that again the several stages of the tomatoes are picked at the plant. However the question is if it would not be better to pick pre-climacteric and to let the samples reach climacteric and post-climacteric stages during storage and not at the plant.
3. For selection of tomatoes we should prepare samples with a maximum coeffi-

cient of variability of ethylene of ± 20%.
This request and request of point 2 make necessary to have a greater amount of tomatoes.

3.1. A greater amount of tomatoes implicates agreements with IVT, to be made now, in order to dispose of larger amounts of plants exclusively for us.

4. The selection of tomatoes in the new harvest would be done:
   a) by size;
   b) by weight;
   c) ethylene production.

   For ethylene production the Net Vol (container minus tomato volume) would be determined experimentally and not by calculation.

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Addendum. During the preparation of this Rapport it was found in the literature that free radical scavengers inhibit ethylene production in fruit slices. (Baker, J.E., M. Lieberman, J.D. Anderson 1978.: Inhibition of ethylene production in fruit slices by a rhizobitoxine analog and free radical scavengers. Plant Physiol. 61, 886-888).
Therefore our hypothesis about free radical effect on ethylene biogenesis has been already demonstrated. Moreover, the article of Baker et al. (loc. cit.) confirms our assumption that studies of Superoxide dismutase and peroxidase should be carried out with slices rather than with acetone powders.

Wageningen, 16-2-1979

Table 1. Moneymaker tomatoes picked at assumed stage of unripe (green colour) for selection via ethylene conc. Date of picking 25-9-1978

number of tomato	weight (g)	ethylene concentration (ppm in 40 mins by 100 g)	tomato for acetone powder	tomato for firmness
1	64.315	0.019	+	
2	85.920	0.144		
3	73.740	0.174		
4	75.750	0.040		+
5	68.610	0.031	+	
6	73.770	0.102		
7	78.200	0.076		
8	73.495	0.054	+	
9	69.365	0.047	+	
10	63.510	0.045		+
11	71.735	0.072		
12	66.195	0.036	+	
13	68.690	0.186		
14	76.475	0.014		+
15	76.865	0.106		
16	61.035	0.078		
17	68.765	0.042	+	
18	60.065	0.094		
19	59.780	0.044	+	
20	61.710	0.062		
21	57.970	0.003		+
22	59.195	0.017	+	
23	55.865	0.063		
24	60.330	0.046	+	
25	53.080	-		
26	56.055	0.059		
27	53.810	0.076		
28	49.950	0.055	+	
29	53.225	0.028		+
30	53.065	0.158		
31	52.195	0.035		+
32	55.815	0.003	+	
33	52.315	0.021	+	
34	50.000	0.030	+	
35	42.000	0.010		+
36	44.195	0.004		+
37	46.295	0.023	+	
38	41.915	0.121		
39	46.685	0.021	+	
40	47.790	0.009	+	
41	50.890	0.071		
42	47.230	0.012		+
43	46.320	0.051		
44	39.205	0.092		
45	43.335	0.010	+	
46	34.740	0.018	+	
47	38.430	0.085		
48	35.630	0.024	+	
49	35.140	0.017	+	
50	35.080	0.044		
51	34.925	0.016		
52	39.645	0.000	+	
53	32.085	0.006		
54	33.595	0.017		
55	32.995	0.011		
56	33.995	0.000		
57	30.505	0.029		

Table 2. Moneymaker tomatoes from Table 1 selected (a) for preparation of acetone powder, and respective ethylene concentration and production. Concentration was estimated directly whereas production was calculated in a later period.

tomato nr.	weight (g)	vol. of tomato(L)	net volume (0.664 L - vol. tomato) (L)	ethylene	
				concentration (ppm in 40 min by 100 g tomato)	production $nL \cdot h^{-1} \cdot g^{-1}$
1	64.315	0.066	0.598	0.019	0.170
5	68.610	0.070	0.594	0.031	0.276
8	73.495	0.075	0.589	0.054	0.477
9	69.365	0.071	0.593	0.047	0.418
12	66.195	0.068	0.596	0.036	0.322
17	68.765	0.070	0.594	0.042	0.374
19	59.780	0.061	0.603	0.044	0.398
22	59.195	0.060	0.604	0.017	0.154
24	60.330	0.062	0.602	0.046	0.415
28	49.950	0.051	0.613	0.055	0.506
32	55.815	0.057	0.607	0.003	0.027
33	52.315	0.053	0.611	0.021	0.192
34	50.000	0.051	0.613	0.030	0.276
37	46.295	0.047	0.617	0.023	0.213
39	46.685	0.048	0.616	0.021	0.194
40	47.790	0.049	0.615	0.009	0.083
45	43.335	0.044	0.620	0.010	0.093
46	34.740	0.035	0.629	0.018	0.170
48	35.630	0.036	0.628	0.024	0.226
49	35.140	0.036	0.628	0.017	0.160
52	39.645	0.040	0.624	0.000	0

Vol. of tomato = weight divided by specific gravity (0.98). Results in ml were converted to L.

(a)This selection, based on ethylene concentration (ppm produced by 100 g tomato in 40 minutes) was between 0 and 0.055.

Table 3. Ethylene production ($nL \cdot h^{-1} \cdot g^{-1}$) of 'rin', F_1 and Moneymaker tomatoes. Ethylene production was calculated for each tomato individually (n = number of individual tomatoes). Afterwards the group was homogenized for preparation of acetone powders that will be used as source of superoxide dismutase and peroxidase activities

date of picking	variety	assumed stage	colour	number of tomatoes picked	number of tomatoes selected (n) [b] and respective values of ethylene production				
					$\bar{n}$	$\bar{x}$	$\bar{s}$	CV (%)	CL (α=0.05)
4-9-1978	rin	-[a]	green	48	6	0.224	0.044	19	0.178 - 0.270
6-9-1978		-[a]	yellowish	35	10	0.078	0.028	36	0.058 - 0.098
25-9-1978	Money-maker	pre-cl.	green	58	21	0.245	0.145	59	0.179 - 0.311
18-9-1978		cl.	green-yel-low/orange	54	20	11.923	2.524	21	10.742 -13.104
14-9-1978		p.-cl.	red	42	20	10.048	1.097	11	9.535 -10.561
30-8-1978	F_1	pr.-cl.	green	73	20	0	0	0	0
21-9-1978		climact.	green-yel-low/orange	70	20	4.131	1.236	30	3.553 - 4.709
11-9-1978		p.-cl.	orange/red	68	20	1.345	0.217	16	1.243 - 1.562

(-)[a] 'rin'tomato does not have a climacterium line

pre-cl. pre-climacterium, cl = climacterium, p-cl. = post-climacterium

[b]) The selection criterion, based on ethylene concentration (ppm produced by 100 g tomato in 40 minutes) was between 0.026 and 0.037 for rin green, 0.005 and 0.020 for rin yellowish; 0 and 0.055 for Moneymaker green, 1.010 and 2.100 for Moneymaker green-yellow/orange, 0.858 and 1.362 for Moneymaker red; 0 and 0.008 for F_1 green, 0.334 and 0.901 for F_1 green-yellow/orange, 0.097 and 0.192 for F_1 orange/red

Table 4. Same as Table 3 with the difference that samples were for firmness measurements instead of preparation of acetone powders. 'rin' was not used for firmness measurements

date of picking	variety	assumed stage (a)	colour (b)	number of tomatoes picked	number of tomatoes selected (n) [c] and respective values of ethylene production				
					n	$\bar{x}$	s	CV (%)	CL (α=0.05)
25-9-1978	Money-maker	pre-cl.	green	58	9	0.192	0.143	75	0.082 - 0.302
18-9-1978		cl.	g/y/o	54	10	10.733	1.233	11	9.851 -11.615
14-9-1978		p.-cl.	red	42	10	11.198	2.521	22	9.395 -13.001
30-8-1978	F_1	pre-cl.	green	73	10	0	0		0
21-9-1978		cl.	g/y/o	70	10	3.932	0.713	18	3.422 - 4.442
11-9-1978		p.-cl.	o/r	68	10	1.249	0.276	22	1.052 - 1.446

(a) pre-cl. = pre-climacterium, cl. = climacterium, p.-cl. = post-climacterium
(b) g/y/o = green/yellow/orange; o/r = orange/red (colours visually determined)

[c]) The selection criterion, based on ethylene concentration (ppm produced by 100 g tomato in 40 minutes) was between 0.003 and 0.045 for Moneymaker green, 1.035 and 1.420 for Moneymaker g/y/o, 0.641 and 1.631 for Moneymaker red; 0 and 0.008 for F_1 green, 0.343 and 0.671 for F_1 g/y/o, 0.078 and 0.173 for F_1 o/r.

Fig. 2. Photo of the flask Triomphe (Verre special, France) where one tomato was incubated for 40 minus at 20°C. A hole of 5 mm diameter covered with a septum allowed that an injection was introduced for removing head space sample (2.5 or 1.0 ml) for GLC.

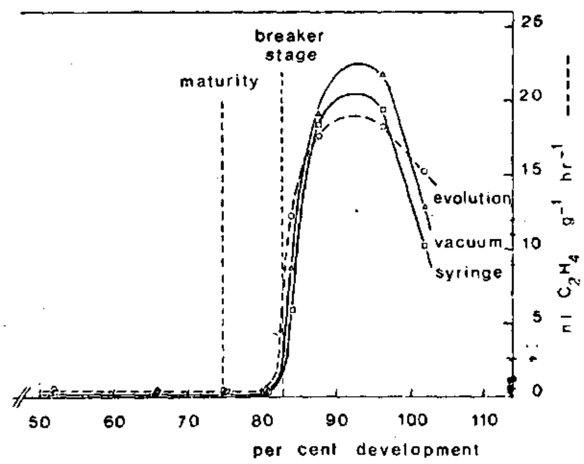

Fig.3 — Ethylene production during the second half of the development of tomato fruit, determined as the evolution from an enclosed fruit or by extraction, either in vacuo or with a syringe. Single *determinations* per *fruit*. (Knegt et al. 1975)