



Tomato pulp quality from transgenic fruits with reduced polygalacturonase (PG)

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The physico-chemical and sensory properties of tomato pulps (i.e. diced tomatoes with 30% tomato juice as packing medium), prepared from transgenic tomato (*Lycopersicon esculentum*) fruits with reduced levels of polygalacturonase (PG) activity due to the expression of a PG antisense gene, were evaluated. The application of genetic modification yields products that have improved viscosity (40–60%), colour (30–40%) and many sensory attributes in comparison with their conventional counterparts. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Among many newly developed tomato products with 'high service content', tomato pulp was one of the first to achieve consumer favour and it still represents the largest share of the market. The demand for high-consistency tomato products has risen markedly and particularly for tomato pulp with a high flavour and a good homogeneous colour.

To meet the new consumer demand, food companies have two routes: (1) innovations leading to an increased range of products, from already known raw materials, and (2) introduction of a new range of raw materials.

One of the techniques that the food industry has available to increase its competitiveness, quality and range of products, is biotechnology. So far, the impact of biotechnology has been limited to food ingredients, including enzymes used as additives or processing aids. Recently, methods have been developed that will also allow the development of foodstuffs with intrinsically modified characteristics. Several of these products are now available in the marketplace. These food products are generated from genetically modified plants. In some instances, enzymes—which play an important role in the determination of food quality attributes—are modified within the food raw material. One of the first products has been genetically modified tomatoes in which the endogenous levels of an enzyme involved in cell-wall polymer metabolism is modified.

Tomatoes are one of the most important crops in the world, not only because of their volume (Moskowitz

and Porretta, 1997), but also because of their overall contribution to nutrition. Tomatoes are consumed either fresh or as a range of processed products. The tomatoes used in these markets must have very distinct quality characteristics. Fresh tomatoes must have acceptable flavour and handling characteristics to satisfy consumer demand and distribution requirements. On the other hand, processing tomatoes must have intrinsic rheological characteristics to make them suitable for various processing applications such as juice, ketchup or sauce production.

Breeding companies have generated a range of tomato varieties aimed at satisfying the above quality criteria. In addition, they provide the farmer with quality germplasm adapted to specific growing environments, thus allowing maximal scale and yield of production.

However, despite the efforts of the tomato-breeding companies, fresh tomatoes do not bear the high flavour quality that European consumers desire and could thus benefit from further improvements. In addition, improved processing characteristics (e.g. firmer dice) are sought by the food companies to stay competitive and provide flexibility during manufacturing. As traditional breeding is a slow process and the incorporation of multigenic traits is complex, novel approaches are required to accelerate the introduction of improved tomato varieties. The production of genetically modified tomatoes with improved processing characteristics offers such an opportunity.

During tomato ripening, many metabolic processes affecting quality take place. These influence the synthesis and action of hormones responsible for the rate of ripening, the biosynthesis and deposition of carotenoids in chromoplasts determining colour pigment production, the metabolism of sugars and acids involved in

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flavour determination, and modifications to the structure and composition of the cell walls affecting fruit firmness and processing characteristics.

These qualitative changes are due to the action of specific enzymes whose gene expression is altered during fruit ripening.

To utilize these genes for quality improvement, methods for the modification of the expression of genes during plant growth and development had to be developed. The methods involve the introduction of genes into the plant genome in which either all or part of the target gene is introduced in either a sense or an antisense orientation (Smith *et al.*, 1988, 1990). Expression of the partial sense or antisense effect genes leads to inhibition of the target gene. This allows the precise targeted modification of single or multiple-effect genes. This approach has been used to improve textural characteristics of tomatoes by inhibiting enzymes involved in cell-wall metabolism.

Texture is a major quality parameter of both fresh and processed tomatoes, and cell-wall structure is a major factor determining processing characteristics, firmness and handling properties. Cell-wall components that contribute to textural characteristics are cellulose, hemicellulose and pectins.

Polygalacturonase (PG) hydrolyses α -1,4 linkages in the polygalacturonic acid component of the cell walls of ripening tomato and has been successfully purified (Tucker *et al.*, 1982). The enzyme is synthesized only during tomato fruit ripening (*de novo*) and is localized within the cell-wall compartment of the tomato pericarp cells. Three isoforms arise from post-translational modification of a single polypeptide derived from a single gene (Bird *et al.*, 1988). The gene encoding PG has been cloned (Grierson *et al.*, 1986; Bird *et al.*, 1988).

Genetically modified tomatoes with reduced levels of the cell-wall-modifying enzyme (PG) have been generated (Smith *et al.*, 1988; Hall *et al.*, 1993; Schuch, 1994). In the first instance, the residual PG enzyme activity after introduction of PG effect genes was approximately 10% of that in unmodified tomatoes (Smith *et al.*, 1988). Later transformed lines expressed PG at < 5%.

It is likely that inactivating PG will decrease the level of pectin depolymerization; higher pectin chain length will have a knock-on effect on processed tomato product quality.

To assess whether these genetically modified tomato lines have improved commercial potential for the processing industry, the quality characteristics of products obtained on a semi-industrial scale have been determined.

MATERIALS AND METHODS

Plant material

Tomato plants supplied by Zeneca Plant Science were grown in fields located in the North of Italy (Parma)

and in the South (Salerno) following consent from the Italian Ministry of Health (consent No. B/IT 95/14) in a randomized complete block design with five replications for each cultivar in each location. Uniformly ripened (80% of the crop), fresh and healthy tomatoes from the field sites, were harvested and transported to the pilot plants.

Sample preparation

Diced tomato formulations were prepared in 50 kg batches; each consisted of mixing diced tomatoes prepared by washing, peeling, dicing (dice 8 mm on a side) and draining, with a partially concentrated (8° Brix) covering juice (70:30, t:t).

The covering juice was prepared with the same tomato variety used for dice, on an experimental line (350 kg h⁻¹) from washed tomatoes by crushing, 'hot break' enzyme inactivation (95°C), sieving (0.8 mm) and concentration to 8° Brix by vacuum heating (62°C, 0.2 bar).

All final products were hot-filled (80°C) in 1 kg cans and then pasteurized in a boiling water tunnel (100°C, 25 min).

Chemical and physical analysis

For each line, subsequently marked by numbers (Table 1), the following determinations were performed (Porretta, 1991a,b, 1992a, 1992).

Drained weight, i.e. all of the product that remains after 30 s draining on sieve with holes of 2.8 mm × 2.8 mm, was determined following the Official Methods of Analysis (Official Italian Methods, 1989).

Total acidity (as citric acid monohydrate, g per 100 g of total solids) was measured by titrating the tomato slurry with 0.1 N NaOH to pH 8.1 with an automatic titrator, pH was measured with a pH meter, and total solids content by oven-drying at 70°C at reduced pressure following the Italian Official Methods of Analysis (Official Italian Methods, 1989).

Colour was determined on the vacuum-sealed homogenized product, using a model XL 800 colorimeter from Gardner Laboratory Division (Bethesda, MD, USA) with the C-C.I.E. illuminant and the BCR (Community Bureau of Reference, Brussels) reference tile ($L = 25.8$; $a_L = 33.9$; $b_L = 14.8$; $a_L/b_L = 2.29$).

Consistency was measured using a Bostwick consistometer (Rossi & Catelli, Parma, Italy) by measuring the flow of the undiluted juice (Bostwick juice) and pulp (Bostwick pulp), as distance travelled (cm) in 30 s.

Volatile acidity (g of acetic acid per kg total solids, TS) and pectins (pectic acids, pectates, protopectins,

Table 1. Identification of the products prepared

Code	Description
1, 3, 5	Control samples
2, 4, 6	Genetically modified samples

expressed as g of galacturonic acid monohydrate, g kg^{-1} TS) were all determined following the Italian Official Methods of Analysis.

Soluble solids (SS, %) were checked on the homogenized product using an RFM81 digital refractometer (Bellingham & Stanley Ltd, Kent, UK).

Glutamic acid (g kg^{-1} TS) was determined by enzymic analysis (Boehringer, Mannheim, Germany).

For sugar determinations a NH_2 column (mean particle diameter $10 \mu\text{m}$, Merck, Darmstadt, Germany) was used with acetonitrile-water (80:20, v/v) as eluent and a differential refractometer (Model 410, Waters Associates, Milford, MA, USA) as detector. The flow rate was 1.5 ml min^{-1} and the injection volume $10 \mu\text{l}$ of the filter-aid filtrate from a homogenized slurry of the whole product.

Peak areas were obtained with an integrator.

Sensory analysis

Sensory tests were carried out by a 7-member panel selected and trained to taste tomato products by assessing acidity, natural taste (characteristic flavour), appearance (homogeneity of redness), bitterness, spoiled taste, fruitiness and viscosity on a 1–9 (nil to extreme) category scale (Porretta, 1992b).

The attributes used have been previously identified (Porretta *et al.*, 1993) as being related to consumer acceptability for this type of product.

Each attribute is related to an hedonic assessment as follows: acidity (1 = not sour at all; 9 = sour), naturalness (1 = taste very similar to fresh tomato; 9 = taste very different from the fresh tomato), viscosity (1 = solid phase completely separated from the liquid phase; 9 = very consistent product), appearance (1 = not homogeneous at all; 9 = fully homogeneous), fruitiness, spoiled and bitter taste (1 = not noticeable; 9 = very strong).

The attributes used by the panel were derived from the main objective in order to obtain a general evaluation of the product.

Preliminary training sessions were conducted to familiarize consumers with the specific sensory attributes.

Assessors were selected from a group of people consisting of experts (6) in tomato products and consumers (6) by progressively excluding those (2 experts and 3 consumers) who were unable to consistently recognize the characteristics required. In each session two single samples were given in random order; samples were assessed at 50°C under artificial daylight illumination (Thorn fluorescent 40 W tubes, 900 lux).

Five panel replications were carried out on each sample.

Polar plotting of parameters (Quantitative Descriptive Analysis, QDA profiles)

This section was designed to contribute to the definition of the quality of tomato pulp and for a qualitative

comparison between genetically modified products and their conventional counterparts.

Although the QDA method is particularly useful for sensory profiles (Stone and Siedel, 1985), it can be applied to qualitative chemical profiles (Porretta, 1991a). In addition, a new method of applying this technique has been previously reported based on multivariate statistical analysis (Porretta, 1994).

In order to derive non-dispersive diagrams where many parameters are involved, only the statistically different ($p \leq 0.05$) were selected.

For each parameter the averages represent the extreme of each polar axis; the angles between the outer lines are derived from the correlation coefficients. The range of each parameter was expressed as a decimal.

For all the characteristics examined the upper limit of the range was defined to correspond to better quality. In order to apply this criterion to all parameters, in the case of the axes relative to Bostwick pulp (5) and Bostwick juice (6) for which the maximum value corresponds to poor quality, the value reported is the difference between 10 and the determined value.

Finally, to adapt the axes of the QDA profiles to the relative importance of the different parameters and, thus, to express an overall and weighted judgement on quality, the length of the axes relative to the a_L/b_L colorimetric ratio (1), drained weight (3), Bostwick pulp (5) and Bostwick juice (6) has been extended by 25% with respect to the other axes (QDA-Stat, 1994). These characteristics were given greater weighting than the others on account of the policy of diced tomato manufacturers to attach greater importance to them (25% was arbitrarily chosen).

Statistical analysis

Three replications were carried out for each experiment.

Data were processed by: analysis of variance (one-way ANOVA, using the Least Significant Difference, LSD, test with 95% confidence limit) to determine the performances of the panel members as well as the differences between the parameters examined.

Principal Components (PCA) and Correlation Analyses were processed with Statgraphics (STSC, 1996).

PCA was carried out in order to build a new system in which the variables are given a loading coefficient proportional to their relative importance. For PCA, each variable was standardized to variance 1.0 before the multivariate analysis to ensure equal probability of all the variables participating in the modelling.

Correlation analysis was carried out to identify the interdependence between the significantly different variables ($p \leq 0.05$) as well as allowing the polar coordinate scales to be represented.

QDA profiles were obtained using QDA-Stat, ver. 2.0 (QDA-Stat, 1994).

RESULTS AND DISCUSSION

The results of the physical, chemical and sensory analysis are given in Table 2.

The most interesting significant linear correlations established between the sensory attributes and the physical and chemical parameters were those between appearance and b_L ($r=-0.88$) and drained weight ($r=0.89$) and viscosity ($r=0.87$) and Bostwick pulp ($r=-0.95$), between natural taste and total acidity ($r=-0.84$) and between viscosity and drained weight ($r=0.87$) and Bostwick juice ($r=0.94$).

From the above strong correlations it was quite easy to confirm the great importance of the rheological parameters, for the products examined.

Another significant correlation normally found in physicochemical tomato data evaluation was between total pectin content and b_L ($r=-0.93$).

The data of Table 2 were also subjected to principal component analysis, a multivariate technique especially effective when the variables are numerous.

The loadings showing the relative contribution of the variables to the first two principal components (PCs) are shown in Fig. 1. The percentage of variance explained is 52% for PC1, and 26% for PC2 and the total variance accounted for by the first three PCs is 87%.

Figure 2 shows the graph of the scores for the first two components built on all the normalized variables. This graph can be easily used as a classification analysis (Piggott, 1988).

Principal component analysis revealed that the 6 tomato lines (with 3 replications each) did fall into different areas on the basis of the variables examined; i.e.

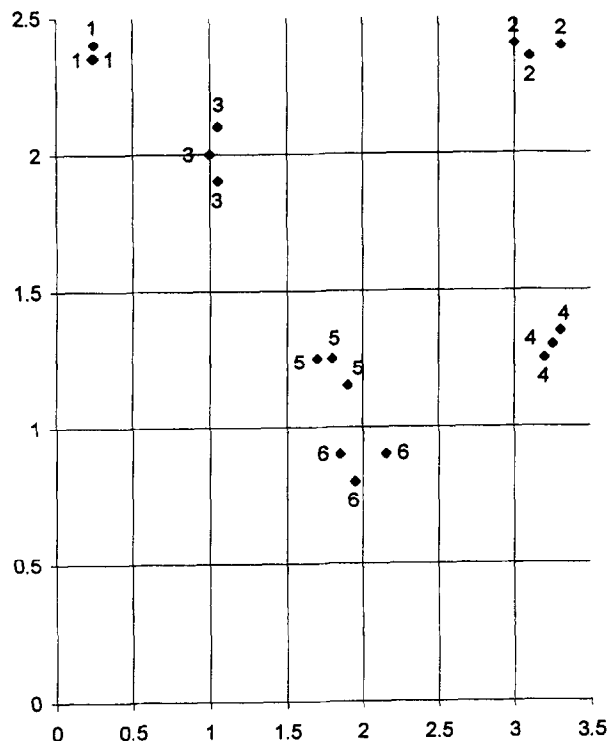


Fig. 2. Scatterplot of the scores of the samples examined on the plane of the first two principal components. The variances accounted for in the first three principal components are, respectively, PC1 = 52%, PC2 = 26%, PC3 = 9%.

the control samples were different from the modified ones, especially in the cases of sample 1-2 and 3-4, while closer (more similar) were samples 5 and 6.

Only those parameters with higher loading coefficients were chosen for QDA profiles. A more complete

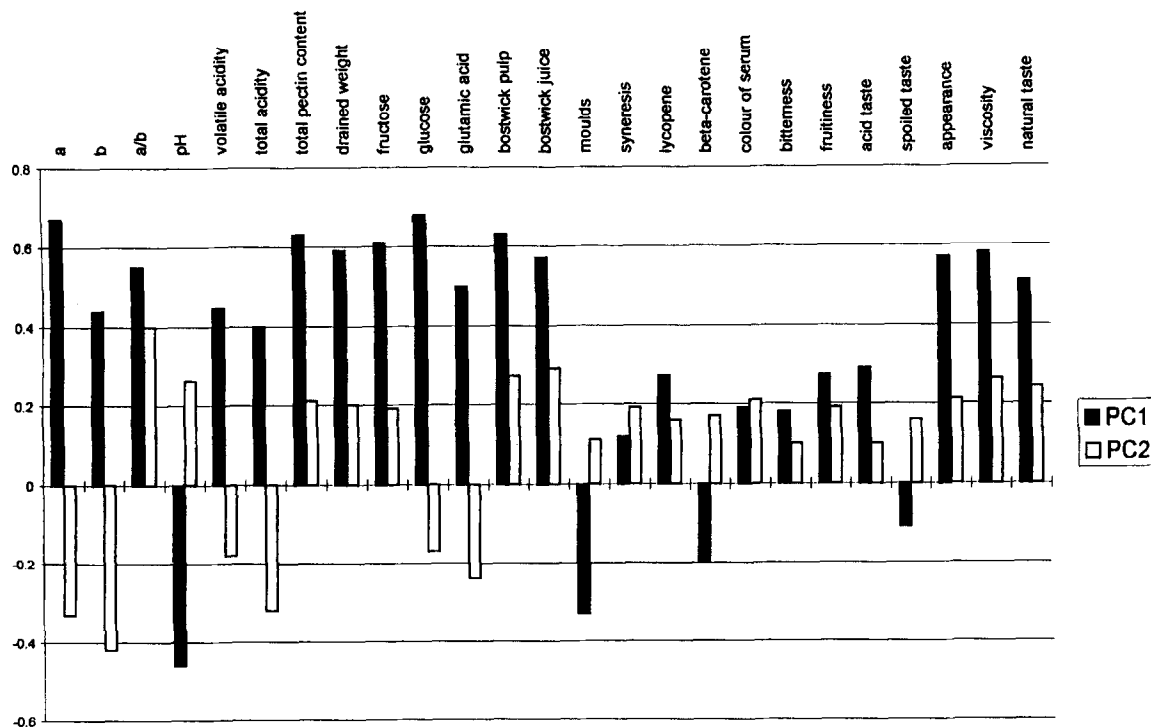


Fig. 1. Loadings of all the variables for the first two principal components.

Table 2. Results of the physicochemical and sensory analyses along with one-way ANOVA results carried out between control and genetically modified data products

	1	2	3	4	5	6
a _L	1.88±0.112	a 1.96±0.096	a 1.98±0.147	a 2.01±0.118	a 2.00±0.098	a 2.11±0.079
b _L	12.4±0.33	a 10.6±0.27	b 14.6±0.27	a 11.5±0.30	b 12.1±0.17	a 10.1±0.21
pH	4.55±0.313	a 4.48±0.418	a 4.51±0.370	a 4.43±0.222	a 4.55±0.432	a 4.53±0.331
Volatile acidity g kg ⁻¹ TS	0.024±0.1×10 ⁻³	a 0.035±0.11×10 ⁻³	b 0.011±0.2×10 ⁻³	a 0.026±0.1×10 ⁻³	b 0.027±0.18×10 ⁻³	a 0.018±0.21×10 ⁻³
Total acidity, %TS	6.21±0.633	a 6.52±0.846	a 6.66±0.411	a 6.47±0.619	a 5.71±0.498	a 5.88±0.337
Total pectin content, %TS	3.76±0.654	a 4.03±0.553	a 3.11±0.518	a 3.69±0.379	a 3.98±0.518	a 4.21±0.312
Drained weight, %	77.2±3.18	a 86.1±2.21	b 81.0±3.27	a 86.7±2.11	b 80.1±3.32	a 89.6±2.24
Fructose, g kg ⁻¹ TS	288±11.10	a 297.5±9.21	a 270.0±18.63	277.1±14.7	251.8±14.33	a 249.6±13.19
Glucose, g kg ⁻¹ TS	244±9.3	a 259.3±7.18	a 223.1±11.14	a 234±10.10	a 209.2±12.30	a 214.6±10.18
Glutamic acid, %TS	4.96±0.103	a 5.05±0.121	a 3.44±0.098	a 3.2±0.070	a 4.32±0.313	a 4.14±0.288
Bostwick pulp (distance in cm in 30 s)	7±1	a 4±1	b 8±0.5	a 3.5±0.5	b 8±1	a 3.5±1
Bostwick juice (distance in cm in 30 s)	9±1.5	a 5.5±1	b 10±1.5	a 5±1	b 11±1.5	a 5±1
Syneresis (%)	45	a 7	b 95	a 9	b 80	a 10
Appearance ^a	4.1±0.8	a 6.3±0.5	b 4.0±0.4	a 5.5±0.6	b 5±0.7	a 7.8±0.6
Viscosity ^a	3.1±0.4	a 7.7±0.6	b 3.7±0.3	a 6.9±0.5	b 2.4±0.4	a 6.4±0.5
Natural taste ^a	6.2±0.2	a 6.8±0.3	b 5.3±0.2	a 5.9±0.3	b 7.1±0.3	a 7.7±0.2
Acidity ^a	3.1±0.5	a 3.4±0.7	a 4.1±0.6	a 4.3±0.7	a 4.4±0.7	a 4.6±0.4
Bitterness ^a	1.2±0.7	a 1.4±0.8	a 1.3±0.7	a 1.9±1	a 1.7±0.9	a 1.1±0.6
Spoiled taste ^a	0.8±0.3	a 1.1±0.3	a 1.2±0.6	a 0.8±0.5	a 1.3±0.7	a 1.4±0.6
Fruitiness ^a	5.4±0.7	a 5.7±0.6	a 6.1±0.5	a 6.0±0.8	a 5.8±0.4	a 5.6±0.6

^aSensory results.

outline of the QDA method used has been reported previously (Porretta, 1991a, 1994).

After determining the significant difference between the products ($p \leq 0.01$), as well as the reproducibility of panellists performances ($p \leq 0.05$), the correlation matrix of the significantly different ($p \leq 0.05$) main analytical data (i.e. with the higher loading coefficients) was calculated (Table 3).

Figures 3–5 show the QDA profiles of the modified and control tomato pulps.

Colour (L , a_L , b_L)

Despite a general/theoretical indication that genetic modification should not practically affect this parameter, a significant benefit is obtained (Zeneca Plant Science, 1996, internal report).

A significant increase of the a_L/b_L ratio was obtained in the modified products; this fact derives from higher b_L values of the control samples rather than from higher a_L values of the modified ones.

The reported results derive from trials carried out using the same harvesting time for both control and modified fruits.

In repeated experiments, statistically significant differences in firmness during later ripening stages were measured. It has also been demonstrated that, despite small differences, damage to tomatoes, both on and off the vine, is reduced. This leads to improved post-harvest handling of low-PG tomatoes and demonstrates that tomatoes with reduced levels of PG activity are firmer than control fruits and can last on the vine for a longer period (unpublished data).

The genetic modification permits the fruit to stay longer in the field than the control samples without spoiling while the lycopene content increases.

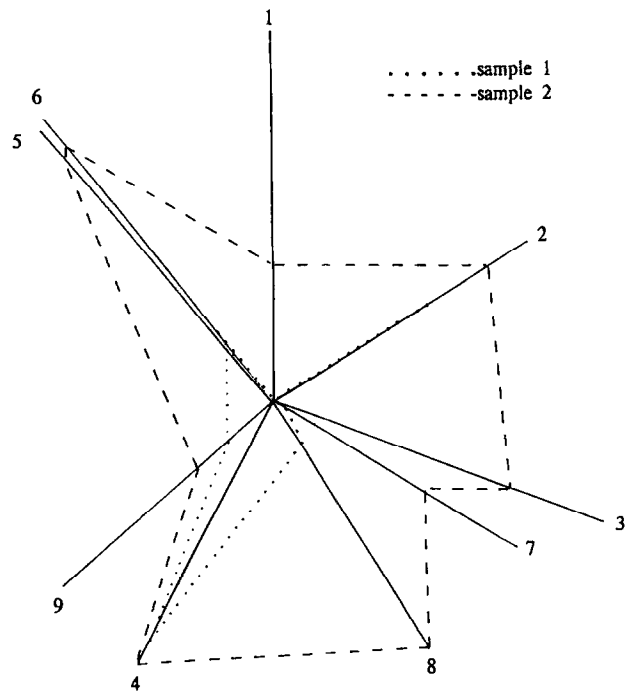


Fig. 3. Comparisons between the profiles of the different products prepared (mean data). Key to polar coordinate scales; 1— a_L/b_L , 2—total pectin content, 3—drained weight, 4—total sugar content, 5—Bostwick pulp, 6—Bostwick juice, 7—appearance, 8—viscosity (sensorial), 9—natural taste (not to scale).

Volatile acidity

The values obtained in general are very low and, even excluding sample 6, volatile acidity in modified tomatoes is significantly higher than control samples. A possible spoilage of the raw material and a consequent acetic acid formation has been excluded. Control samples in general have a very low impact flavour. An

Table 3. Correlation matrix of the analytical data ($p \leq 0.05$) for the research of linear interdependence. a_L/b_L , b_L , pH, volatile acidity, total acidity, total pectin content, drained weight, fructose, glucose, glutamic acid, Bostwick pulp, Bostwick juice, appearance, viscosity (sensorial), natural taste, spoiled taste, acid taste, bitterness, fruitiness

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1.00																		
2	-0.44	1.00																	
3	-0.13	0.17	1.00																
4	-0.28	-0.59	-0.26	1.00															
5	-0.37	0.40	-0.64	-0.09	1.00														
6	0.37	-0.93	0.17	0.59	-0.67	1.00													
7	0.79	-0.70	-0.54	0.11	0.02	0.45	1.00												
8	-0.74	0.02	-0.40	0.50	0.71	-0.18	-0.18	1.00											
9	-0.61	-0.18	-0.40	0.58	0.62	0.01	-0.01	0.97	1.00										
10	-0.45	-0.37	0.50	0.57	-0.26	0.54	-0.26	0.40	0.49	1.00									
11	-0.48	0.81	0.63	-0.36	-0.11	-0.53	-0.89	-0.17	-0.33	0.05	1.00								
12	-0.44	0.75	0.65	-0.30	-0.21	-0.44	-0.87	-0.23	-0.39	0.06	0.99	1.00							
13	0.77	-0.88	-0.16	0.22	-0.34	0.76	0.89	-0.29	-0.08	0.10	-0.80	-0.75	1.00						
14	0.38	-0.66	-0.74	0.37	0.37	0.34	0.87	0.34	0.48	-0.05	-0.94	-0.94	0.70	1.00					
15	0.63	-0.68	0.47	0.12	-0.84	0.83	0.40	-0.62	-0.45	0.34	-0.26	-0.17	0.75	0.07	1.00				
16	0.30	0.25	0.74	0.78	0.77	0.50	0.45	0.40	0.50	0.33	0.22	0.18	0.18	0.38	-0.40	1.00			
17	0.28	0.87	0.79	0.69	0.56	0.41	0.28	0.19	0.21	0.11	0.17	0.22	0.27	0.35	-0.46	0.49	1.00		
18	0.46	0.42	0.56	0.21	0.55	0.44	0.50	-0.58	-0.61	-0.52	0.45	0.55	0.43	0.47	-0.69	0.59	0.47	1.00	
19	0.58	-0.35	0.68	0.11	0.31	0.66	0.57	0.76	0.79	0.70	0.20	0.17	0.60	0.14	0.79	-0.58	-0.60	-0.68	1.00

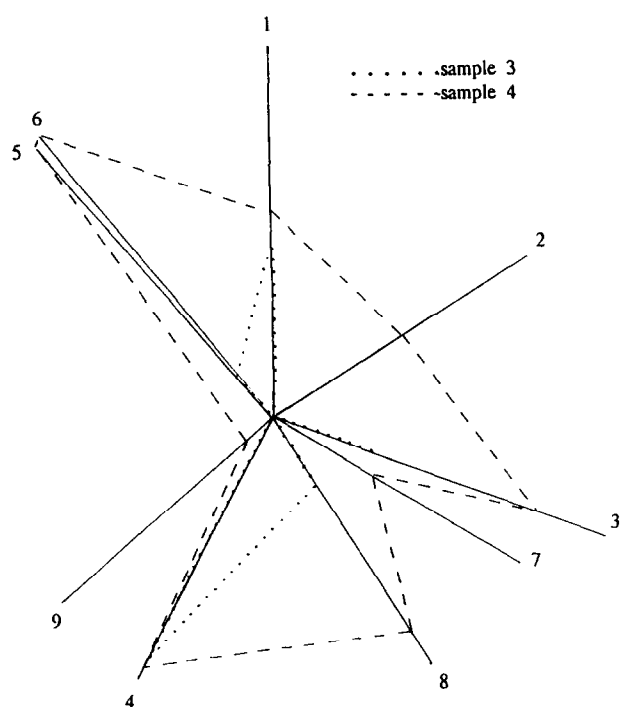


Fig. 4. Comparisons between the profiles of the different products prepared (mean data). Key to polar coordinate scales; 1— a_L/b_L , 2—total pectin content, 3—drained weight, 4—total sugar content, 5—Bostwick pulp, 6—Bostwick juice, 7—appearance, 8—viscosity (sensorial), 9—natural taste (not to scale).

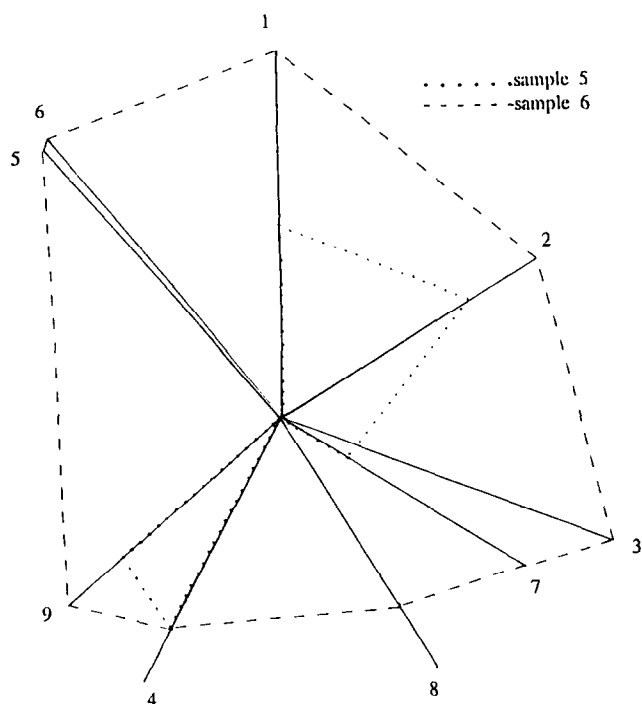


Fig. 5. Comparisons between the profiles of the different products prepared (mean data). Key to polar coordinate scales; 1— a_L/b_L , 2—total pectin content, 3—drained weight, 4—total sugar content, 5—Bostwick pulp, 6—Bostwick juice, 7—appearance, 8—viscosity (sensorial), 9—natural taste (not to scale).

additional paper will report the effects of more than 300 volatile compounds on quality of modified tomato pulp.

Total acidity and pH

Excluding sample 12/3 (only for total acidity), no significant differences were observed in the products prepared.

Drained weight and syneresis

Drained weight and syneresis were significantly affected by the genetic modification.

As shown in Table 2, the drained weight of the modified products was from 6 to 10% greater than the control.

Syneresis was detected by measuring the volume of serum separated from the covering juice after pouring the products through the sieve used for drained weight determination. It was practically absent in all modified products whereas the control tomato pulp gave values of serum syneresis ranging from 45 to 95 ml kg⁻¹ of product.

Pectin content

Total pectin values for the genetically modified tomato pulps were found to be statistically higher ($p \leq 0.05$) than for control products.

The benefits obtained in both drained weight and pectin content are in agreement with other papers published (Smith *et al.*, 1988, 1990).

The average pectin chain length in genetically modified tomatoes is considerably greater than in control tomatoes. This is consistent with the biological role of PG, to reduce pectin chain length in the last ripening phase, which leads to a solubilization of the middle lamellar fraction of the tomato cell wall.

The increase in firmness, i.e. drained weight in the case of pulp, is therefore attributable to the changes in the average molecular weight of tomato cell-wall pectins in fresh tomatoes with low levels of PG.

Reducing sugars and glutamic acid

No significant differences were observed for these parameters between the control and modified tomato pulp. The ratio fructose/glucose ranged typically from 1.15 to 1.21.

This fact is of particular relevance for nutritional evaluation of modified fruits.

The PG enzyme is normally present as an extremely small proportion of the total weight of the tomato fruit. The protein does not contain a preponderance of amino acids which might be considered to be of nutritional value. Thus a reduction in the level of the enzyme does not directly affect the nutritional quality of the fruit.

CONCLUSIONS

It is evident from the data presented that the reduction of PG activity leads to many desirable characteristics of processed products from tomato fruit.

The benefits of low PG tomatoes to the processing company include, not only cost savings from increased product yield, but also enhancements in product quality. A much broader range of benefits can also be realized by the grower, the consumer and the environment. Thus, basic research leading to a better understanding of the tomato-ripening process is being translated into a broad range of potential benefits, which can only be realized fully once genetically modified tomatoes reach the marketplace.

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