**Estudio de caracteres de calidad en un programa de Mejora Genética de variedades tradicionales de tomate del sureste español.**

## PRÓLOGO

Este documento se ha elaborado siguiendo la normativa de la Universidad Miguel Hernández de Elche para la "Presentación de Tesis Doctorales como un conjunto de publicaciones", y se ha dividido en las siguientes partes:

1.- Una *Introducción*, en la que se presenta el tema de la tesis y los antecedentes del trabajo realizado.

2.- Un apartado de *Publicaciones*, que incluye los artículos publicados y un artículo aceptado en el momento de depósito de la tesis.

3.- Un apartado de *Resumen de Resultados, Discusión y Conclusiones*.

4.- Un apartado de *Bibliografía*.

Este documento no incluye un apartado de *Materiales y Métodos*, puesto que se recogen en las publicaciones. En el apartado de *Bibliografía* se reseñan todas las referencias que aparecen citadas en el texto, aunque también lo estén en los artículos.

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## 1.- INTRODUCCIÓN

El tomate pertenece a la familia de las Solanáceas, siendo conocido actualmente por *Solanum lycopersicum* (Peralta *et al.*, 2008) tal y como fue denominado originalmente por Linneo en 1753. Dentro de la familia de las Solanáceas encontramos más de 3000 especies, e incluidas en la sección *Lycopersicon* tenemos 12 especies relacionadas, aparte de *S. lycopersicum* o tomate cultivado (Bai y Lindhout, 2007). Estas especies presentan caracteres específicos que han sido detenidamente estudiados y utilizados para mejorar el tomate (Stevens y Rick, 1986).

Se trata de una especie originaria de la región andina, que comprende hoy día parte de Chile, Bolivia, Ecuador, Colombia y Perú (Sims, 1980). A pesar de ello debió ser domesticado posiblemente en México. A su llegada a Europa en el siglo XV fue considerado, en principio, únicamente como ornamental salvo en España e Italia, países donde tempranamente formó parte de la alimentación humana. Es a partir de siglo XVIII o principio del XIX cuando tiene lugar una domesticación mucho más intensa y se extiende su consumo ya por todo el continente europeo.

El tomate tiene muchas características que lo convierten en una planta modelo particularmente interesante como son sus frutos carnosos, raíz simpodial, hojas compuestas, etc., que otras plantas como el arroz o *Arabidopsis* no tienen. Muchas de estas características son agronómicamente importantes y no pueden ser estudiadas con ninguna otra planta modelo (Kimura y Sinha, 2008).

### 1.1. Importancia económica del tomate

Actualmente, el tomate constituye una de las hortalizas de mayor difusión mundial. A ello contribuye el hecho de que se trata de un producto destinado a ser consumido en fresco o bien procesado de múltiples maneras (Costa y Heuvelink, 2005).

A nivel mundial, España se sitúa como el octavo país productor de tomate con, según datos de 2007, 4.081.477 toneladas (MARM, 2009). De hecho, en nuestro país la producción de tomate representa la mayor de todas las hortalizas cultivadas. Una parte importante de esta producción, 884.244 t. es exportada (MARM, 2009), en su mayoría a otros países de la Unión Europea. Otro destino fundamental de este producto es la industria transformadora, que acapara más de la mitad de las ventas (1.656.396 t.), (MARM, 2009).

El valor nutricional del tomate no bastaría para justificar su elevado consumo pues únicamente su contenido en licopeno le aporta un protagonismo especial. Sin embargo, se trata de una hortaliza fundamental y ofrece una amplia diversidad de frutos en cuanto a forma, coloración, tamaño, sabor, etc.

## 1.2. Programa de Mejora

Desde 1998 se viene desarrollando un Programa de Mejora (figura 1) encaminado a posibilitar la recuperación del cultivo de las variedades tradicionales de tomate de la zona del sureste español. Parte del trabajo de dicho programa se recoge en un artículo de divulgación (García-Martínez *et al*., 2008). Este programa es llevado a cabo mediante selección asistida por marcadores moleculares. Como resultado preliminar se ha obtenido una colección de líneas de mejora resistentes para distintos tipos tradicionales que son cultivadas por agricultores que colaboran en dicho programa.

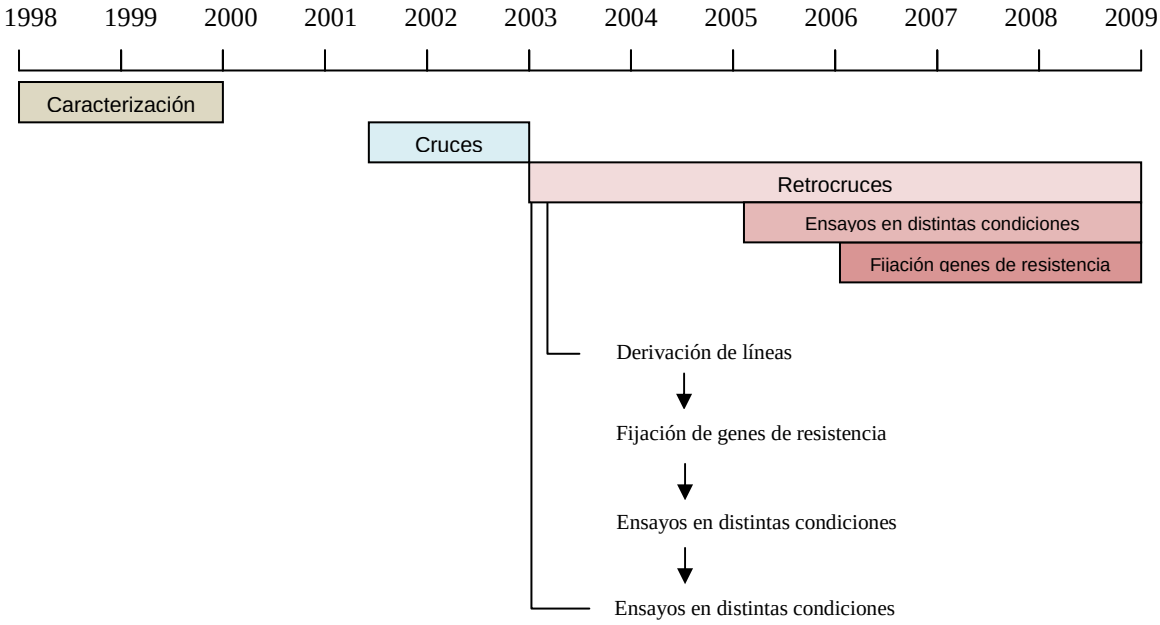


**Figura 1.** Fases del Programa de Mejora Genética bajo estudio.

### 1.2.1. Las variedades tradicionales

Las variedades tradicionales son el resultado de la selección realizada por los propios agricultores en sus cultivos al escoger las mejores plantas con la finalidad de obtener de sus frutos la semilla para la próxima campaña. Estas variedades son pues el resultado del trabajo de selección y mejora realizado por los agricultores (García, 1999; Guzmán *et al.*, 2000; Cebolla, 2005) y se hallan inmersas en el proceso coevolutivo, que les otorga un carácter dinámico y diverso (González y Guzmán, 2006). De esta manera, el criterio tenido en cuenta para la selección, respondía fundamentalmente a la rusticidad del cultivo, así como a otro tipo de aspectos, relacionados más bien con la calidad del fruto. Con los años se ha constituido de esta forma una serie de grupos varietales bien adaptados a cada ambiente y con productos muy apreciados en los mercados locales a los que se destinaban.

No obstante, con la llegada al mercado de los híbridos, desarrollados por empresas para lograr un incremento importante de las producciones, y mejorar de paso la apariencia externa, la homogeneidad de los frutos e incorporar resistencias a plagas y enfermedades, así como posteriormente permitir una

mayor durabilidad de estos frutos, los agricultores fueron sustituyendo sus semillas tradicionales por estas otras comerciales.

Las variedades tradicionales se vieron desplazadas y han pasado a infrautilizarse o incluso puede haberse llegado a su desaparición en algunos casos. Por ejemplo, según datos de la FAO, Estados Unidos ha perdido el 95% de las variedades de col, el 91% de las de maíz, el 94% de las de guisante, el 86% de las de manzana y el 81% de las variedades de tomate cultivadas en el último siglo (http://www.fao.org, consultado en noviembre 2009).

La Comunidad Valenciana es, de entre todas las regiones de la Unión Europea, de las áreas con un patrimonio agrícola más rico y diversificado. Las semillas, además de un recurso, son parte de nuestra cultura e historia (Sánchez, 2008). Muy estrechamente ligado al patrimonio genético que constituye la biodiversidad agrícola valenciana, y además de su valor estrictamente económico, debemos considerar también el patrimonio cultural formado por todo el conjunto de conocimientos y "saber hacer" agrícola que han caracterizado desde muy antiguamente al agricultor valenciano como uno de los mejores practicantes del arte de la agricultura (Nuez y Ruiz, 1999).

Aunque aparentemente vivimos una auténtica explosión de variedades, éstas corresponden a una escasa diversidad genética (Comwell *et al.*, 2001). Este hecho implica una grave pérdida pues la diversidad significa seguridad frente a enfermedades, plagas y condiciones climáticas inesperadas (Cooper *et al.*, 1992). Para utilizar una mayor proporción de la diversidad genética disponible una posible alternativa es volver a distribuir semillas de variedades tradicionales (y especies silvestres relacionadas) a los agricultores, así como incorporarlas a programas de mejora. Estos programas producirían variedades tradicionales mejoradas, que deberían ser ensayadas en distintas condiciones para comprobar su adaptación a los sistemas de cultivo, como paso previo hacia un sistema de mejora integrado. En definitiva se trata de acciones con la finalidad de permitir una mayor posibilidad de elección de variedades para los agricultores (Cooper *et al.*, 1994).

La problemática asociada al empleo de las variedades tradicionales abarca su reducida productividad, la heterogeneidad en sus frutos, así como su carácter perecedero y, fundamentalmente, la susceptibilidad que presenta su cultivo a todas de las virosis que pueden incidir, y ha desembocado finalmente

en arrinconar estas variedades tradicionales hasta convertirlas en una opción inviable muy frecuentemente en la actualidad.

Por otro lado la mejora a menudo ha perseguido objetivos de rendimiento, tamaño del fruto, ausencia de defectos y resistencia a enfermedades, mientras que la calidad organoléptica no siempre se ha tenido en cuenta (Lisanti *et al.*, 2008). Frecuentemente se constatan quejas por parte de los consumidores con respecto a dicha calidad en los tomates comprados en supermercados (Baldwin *et al.*, 2000). Proporcionar mejor sabor a frutas y hortalizas probablemente aumente su consumo, por lo que es necesario prestar más atención al sabor y la calidad nutricional, aunque estos aspectos son mucho más complejos que otros factores de la calidad (Kader, 2008). El consumidor occidental quiere productos de calidad libres de contaminantes y exige algo más que una buena presentación (Nuez, 1995). Las frutas y hortalizas se convertirán cada vez más en productos de una mayor especialización (Brueckner, 2006). En este marco, la posibilidad de recuperar la calidad organoléptica de las variedades tradicionales tiene sentido, pero continúan existiendo importantes impedimentos al cultivo de estas variedades. El tomate hospeda más de 200 especies de un gran abanico de plagas y patógenos que generan significativas pérdidas económicas, pero la naturaleza también nos ha provisto de una gran variedad de resistencias, disponible en las especies silvestres (Bai y Lindhout, 2007). La investigación pública debería prestar su atención a la mejora de algunas variedades locales excelentes que, por presentar un volumen de negocio limitado, quedan fuera de los programas de mejora de las grandes empresas productoras de semillas (Nuez, 1995).

### 1.2.2. Objetivos del Programa de Mejora

El objetivo planteado por nuestro equipo consistió, por tanto, en incorporar resistencia a tres de las virosis más frecuentes en esta zona (Comunidad Valenciana) y para las que se conocen los genes que confieren resistencia o tolerancia a las plantas. Las virosis en cuestión son el *Tomato Mosaic Virus* o mosaico del tomate (ToMV), el *Tomato Yellow Leaf Curl Virus* o virus de la cuchara (TYLCV) y el *Tomato Spotted Wilt Virus* o bronceado del

tomate (TSWV). Para acortar la duración del Programa de Mejora, en lugar de obtener la resistencia/tolerancia directamente de las especies silvestres donde se encuentran los genes de interés, se recurrió a una fuente más cercana, un híbrido comercial, para introgresar estas características.

### 1.2.3. Selección asistida por marcadores moleculares

Los mejoradores pueden hacer uso de la asociación conocida entre un marcador molecular y un rasgo o un segmento del cromosoma para seleccionar fundamentándose en la presencia del marcador molecular, en lugar de tener que comprobar el fenotipo. Este procedimiento se conoce como “selección asistida por marcadores moleculares” (Bai y Linhout, 2007). Entre las muchas ventajas que aportan los marcadores moleculares a un programa de mejora encontramos, por ejemplo:

a) que pueden dar información acerca de la característica que nos ocupa incluso en un estado precoz de desarrollo,

b) son “neutros” o independientes del fenotipo,

c) para poder utilizarlos basta con una pequeña cantidad de material vegetal, y

d) muchos son codominantes, es decir que permiten distinguir los tres genotipos posibles de un gen (Cubero, 1999).

En el Programa de Mejora que nos ocupa, ésta ha sido una herramienta de gran utilidad pues se han empleado marcadores inicialmente ya descritos, y con posterioridad nuevos marcadores desarrollados específicamente para nuestro material, pues en algún caso surgió esa necesidad.

## 1.2.4. Fases del Programa de Mejora

### 1.2.4.1. Caracterización agronómica de las variedades tradicionales

La caracterización y conservación de las variedades locales es uno de los objetivos principales en su preservación y utilización (Ercolano *et al.*, 2008). Estos cultivares tradicionales presentan en general una excelente calidad organoléptica y atesoran una amplia variabilidad genética (Adalid *et al.*, 2008), que los conforma como una interesante fuente de variabilidad para la mejora de la calidad interna de los cultivares modernos (Valcarcel, 2009; Saha *et al.*, 2009).

Teniendo en cuenta el gran esfuerzo y la duración de un programa de mejora se entiende la importancia crítica de la etapa de caracterización, pues hay que asegurarse bien de cómo es el material de partida. Según el *International Plant Genetic Resorces Institute* (IPGRI) en una de sus publicaciones, hay 3 niveles de datos:

1) datos de pasaporte para identificar la procedencia de la muestra,
2) datos de caracterización propiamente dicha, para identificar la muestra y,
3) datos de evaluación para características específicas como resistencia a enfermedades, respuesta a ciertos tipos de estrés, etc. Para los datos de caracterización se dispone de “descriptores” para homogeneizar la información (IPGRI, 1996).

En el caso del programa de mejora desarrollado por nuestro grupo de investigación, la caracterización tuvo lugar durante varios años (del 1998 al 2000). Se evaluaron distintas accesiones en diferentes ambientes y durante varias campañas. Las primeras caracterizaciones llevadas a cabo se recogen en los Trabajos de Fin de Carrera de Ingeniero Técnico Agrícola de Santiago García (1998), ***Caracterización de variedades tradicionales de tomate tipo "Pera”*** y Aránzazu Alonso (1998) ***Caracterización de variedades tradicionales de tomate tipo "Muchamiel"***.

1.2.4.2. Realización de los cruces

Tan importante como la caracterización de las variedades tradicionales a mejorar es la elección de la variedad que será la fuente de los genes de resistencia en nuestro programa de mejora. En este caso recurrimos al híbrido comercial F1 Anastasia, de *Séminis Vegetable Seeds* que contiene los genes: *Sw-5*, que confiere resistencia al virus del bronceado (TSWV), *Ty-1*, que le otorga tolerancia al virus de la cuchara (TYLCV) (Pérez de Castro *et al.*, 2007) y *Tm-2ª* que le proporciona resistencia al virus del mosaico (ToMV).

Los cruces se realizaron de forma manual y el porcentaje de frutos cuajados que producen semillas dependió de distintos factores (la posición de la flor en el ramillete, las condiciones de temperatura, iluminación, humedad, etc.). En nuestras condiciones este porcentaje de frutos cuajados osciló entre el 10 y el 40% (García-Martinez, 2006).

1.2.4.3. Realización de retrocruces

Después de realizar los cruces tendremos que cruzar de nuevo por la variedad tradicional (retrocruzar) para recuperar al máximo posible sus características manteniendo de forma simultánea la resistencia/tolerancia del híbrido.

Para poder conocer en cada generación los individuos portadores de los genes de resistencia/tolerancia con los que continuar los retrocruces hemos empleado marcadores moleculares ligados a ellos. La obtención de la información sobre la presencia o no de cada uno de los genes que deseamos mantener, tradicionalmente se conseguía observando la respuesta fenotípica de las plantas ante la inoculación del virus. La selección fenotípica presenta multitud de inconvenientes, por ejemplo que la sintomatología no siempre es clara (debido principalmente al efecto del ambiente), que se pueden producir escapes en la inoculación del virus, que obliga a manejar de forma adecuada poblaciones del insecto vector, etc. El empleo de marcadores moleculares ligados a los genes de resistencia evita muchos de estos inconvenientes, pero

la selección fenotípica sigue siendo necesaria. En cada generación se hace un seguimiento individualizado de las plantas para, aunque el marcador las indique como resistentes/tolerantes, eliminar aquellas que manifiesten síntomas de alguna de las virosis. Sin embargo, no es posible para todas las virosis llevar a cabo la selección fenotípica en todos los ciclos de cultivo. Simultáneamente, tiene lugar una selección en base a caracteres deseables, tanto agronómicos como de forma de los frutos (aspecto muy característico de los tipos varietales que centran nuestro programa de mejora) y sobretodo organolépticos.

La utilización de invernaderos nos permite realizar dos retrocruces al año, aprovechando tanto el ciclo de invierno-primavera como el de verano-otoño. Si bien es cierto que, al no contar con calefacción, no se dispone de mucho tiempo de pausa entre ambos ciclos (García-Martinez, 2006).

#### 1.2.4.4. Selección asistida por marcadores

La existencia de marcadores moleculares ligados a los genes de interés permite realizar una selección genotípica (porque se basa en una diferencia en la secuencia de ADN) e indirecta (porque se selecciona el marcador, no el gen). Al tratarse de una selección genotípica se dejan de lado los efectos que el ambiente, el manejo de los vectores y la posibilidad de escapes puedan tener. Además, estos marcadores permiten una selección precoz, en semillero, de los individuos resistentes/tolerantes con lo que se transplantan únicamente los individuos de interés con las consiguientes ventajas de ahorro de espacio y tiempo. Se facilita así el trabajo con un elevado número de plantas, necesario para obtener suficientes individuos con los tres genes de resistencia/tolerancia que pretendemos incorporar a nuestras líneas, tras la evaluación con los marcadores.

Sin embargo, el uso de marcadores presenta también una serie de inconvenientes como son su coste, la dificultad en su puesta a punto y la posibilidad de errores por recombinación. Las ventajas superan con creces estos aspectos negativos, por lo que el uso de marcadores en mejora es muy extenso.

Cuando comenzó el programa de mejora se emplearon marcadores previamente descritos por otros autores y los resultados de cosegregación obtenidos con los tres marcadores utilizados fueron muy buenos. Sin embargo, dos de ellos no se comportaban como codominantes con nuestro material vegetal, por lo que se decidió desarrollar nuevos marcadores (García-Martinez, 2006). El empleo de marcadores no debe suponer el abandono de la evaluación de los síntomas en las plantas, pues podrían tener lugar fenómenos de recombinación entre el marcador y el gen o bien podrían producirse errores en el proceso de genotipado.

### 1.3. Puesta a punto de técnicas para determinar la calidad

La propia dinámica del mundo en que vivimos está exigiendo, de forma cada vez más acentuada, productos de mejor calidad. Este fenómeno se observa muy acusadamente en el consumo de productos alimenticios. Esta situación obliga pues a una constante mejora general de la calidad de frutas y hortalizas para poder competir con éxito en estos mercados, cada vez más selectivos. Al existir un exceso de oferta en general, y a lo largo de los doce meses del año, es lógico que el consumidor, cada día mejor informado, exija e incluso esté dispuesto a pagar por frutas y hortalizas de mayor calidad. La calidad incluye, no solamente la que intrínsecamente tienen los productos en el momento de ser empaquetados en origen, sino la que presentan en el momento de ser comprados, y aún más, en el de ser consumidos (Sánchez, 1993). En ocasiones un determinado producto cuenta con un valor añadido, este es el caso de los productos de Agricultura Biológica. Cabe destacar en este sentido que las variedades tradicionales pueden ocupar también un lugar preponderante dentro de este tipo de agricultura. El consumidor de productos biológicos desea calidad y estas variedades se la ofrecen (Nuez, 1995), a pesar de que en ocasiones su aspecto externo no resulte demasiado atractivo.

La calidad es un término difícil de definir objetivamente, ya que es el resultado de un juicio meramente subjetivo y así podemos entender que se apliquen distintos criterios según, entre otros aspectos, si la persona que lo valora es consumidor, comerciante o agricultor (Lavilla, 1998). Además la

calidad de los productos alimenticios es “compleja” porque no puede ser determinada por una sola propiedad o factor aislado, sino por la combinación de todas sus propiedades físicas, químicas y organolépticas, y es “relativa” porque debe ser tal que determine la aceptación por parte del consumidor (Pretel *et al.*, 1993). Los factores de calidad externa son simples (forma, color…) y pueden ser evaluados fácilmente, mientras que los de calidad interna son más difíciles de medir (Roselló y Nuez, 2006). En lo referente a la calidad externa podemos matizar que el tamaño no es en sí mismo un índice de calidad, pero influye enormemente en la calidad comercial del fruto (Suslow y Cantwell, 2005).

Para la evaluación de la calidad podemos efectuar una serie de determinaciones analíticas relacionadas. Podemos comenzar determinando los compuestos mayoritarios (proteínas, grasas, etc.), luego los parámetros organolépticos (aromas, acidez, textura, etc.) y por último otros componentes minoritarios (vitaminas, aminoácidos, antioxidantes, etc.). Finalmente también resultaría interesante la determinación de otro tipo de parámetros que corresponden a la interacción de varios de los anteriores como serían la madurez, la vida útil y los sólidos solubles, por ejemplo (Maqueira y Puchades, 2006).

En estudios de mejora han de analizarse un gran número de muestras en períodos de tiempo bastante cortos aunque también teniendo en cuenta el tipo de almacenamiento que será necesario hasta el momento del análisis del último fruto (Maqueira y Puchades, 2006). En nuestro caso, éste fue el punto donde encontramos el primer obstáculo: conseguir un elevado número de frutos lo más homogéneos posible y en el estado de madurez óptimo para la realización de los análisis, supone un serio inconveniente, especialmente en el caso de las variedades tradicionales, ya que, remontándonos a la fase de campo, llevar a término su cultivo en condiciones de acusada incidencia de virus supone todo un logro.

### 1.3.1. Calidad en tomate

El concepto de calidad en el caso del tomate recibe un tratamiento totalmente distinto según sea el uso al que se destina. Así pues queda bien diferenciada la calidad aplicada al tomate para consumo en fresco y la del tomate destinado a industria, e incluso podemos ir más allá y, dentro de este último grupo distinguir la existencia de tomate concentrado, deshidratado, triturado, enlatado entero o en trozos, pelado o sin pelar, etc. (Llácer *et al.*, 2006). Del mismo modo, actualmente se pueden diferenciar dos grupos principales de material vegetal de tomate para consumo en fresco en función de su calidad organoléptica, las variedades tradicionales y los cultivares modernos (Valcarcel, 2009).

Podemos agrupar los atributos de calidad de la siguiente manera:

- Calidad Organoléptica o Sensorial. Este término podemos a su vez desglosarlo en la fácilmente perceptible calidad externa referida al aspecto externo: tamaño, forma, color, ausencia de defectos, y la más compleja calidad interna que abarca textura y *flavor*, concepto este último que resulta de la combinación del gusto, el olor y el aroma (Lavilla, 1998).
- Calidad Nutricional. Es la capacidad de los alimentos de proporcionar los elementos nutritivos que se necesitan para una buena salud.
- Calidad Poscosecha. Incluye la capacidad de conservación y la resistencia a la manipulación y el transporte.

Ésta es solo una de las posibles clasificaciones del carácter “calidad” y no incluye todos los tipos, pues también podemos referirnos a una calidad sanitaria, calidad para la transformación industrial, calidad legal, etc. (Llácer *et al.*, 2006), pero, para el caso que nos ocupa, esta sencilla diferenciación resulta muy útil.

#### 1.3.1.1. Calidad organoléptica o sensorial

Este término se refiere fundamentalmente a las sensaciones que experimentamos al probar o consumir un alimento y se relaciona con aspectos gustativos, olfativos y táctiles (Valcarcel, 2009). Este aspecto de la calidad incide directamente en la reacción del consumidor frente al alimento (Casañas y Costell, 2006). Intervienen, por lo tanto, en esta valoración tanto aspectos del propio producto como su composición química y nutricional, sus características físicas y estructurales, así como otra serie de factores ligados al entorno y a la propia persona que evalúa el alimento, como por ejemplo su condición cultural y sociológica así como su estado fisiológico y psicológico (Costell, 2005).

El color es un atributo muy significativo en los tomates frescos y en los productos que se obtienen de él. Está influenciado por el estado de madurez en el momento de la recolección y es el resultado de una mezcla compleja de carotenoides. El contenido total de carotenoides aumenta al avanzar la madurez del fruto (Reineccius, 1998). El licopeno es el responsable del típico color rojo en el tomate, pero no todos los tomates tienen licopeno ni todos los tomates son rojos. Dada la gran variabilidad de frutos en el caso del tomate (foto 1) podemos encontrar otras coloraciones como las de un grupo de variedades, de frutos morfológicamente similares al “Muchamiel” y tradicionales de las sierras del sureste de España, que presentan al madurar un color rojo oscuro, en algunos casos casi morado por lo que se les conoce como tomates “Morunos” (García-Martínez, 2006).

**Foto 1.** Diversidad de frutos de tomate

La forma en ocasiones es también un carácter identificativo en ciertos tipos varietales de tomate, especialmente en variedades tradicionales. Así, por ejemplo, podemos encontrar el tipo varietal "De la Pera" formado por un conjunto de variedades que tienen en común una cierta forma aperada de sus frutos; el tipo varietal "Muchamiel" que toma el nombre de la misma localidad, cercana a Alicante, y que está formado por un conjunto de variedades que tienen el fruto aplastado, más o menos rizado y de mayor tamaño que el anterior tipo; el "Tres cascos" de Elche, cuyos frutos tienen forma aperada con tres lóbulos o "cascos" fuertemente marcados y tamaño un poco mayor que el del tipo "De la Pera"; el "Valenciano", un tipo varietal tradicional de Valencia cuyos frutos tienen forma acorazonada o la variedad tradicional "Flor de Baladre", de Murcia, cuyo fruto es similar en forma y tamaño al "Muchamiel", siendo más acostillado o "rizado" (García-Martínez, 2006).

La textura también tiene su importancia y puede implicar que un tomate blando se perciba más intenso en cuanto a *flavor* que uno más duro (Baldwin *et al.*, 2000). El mucílago o gel tiene un papel muy importante en la percepción de la jugosidad del tomate (Valcarcel, 2009).

Respecto al *flavor* encontramos dos definiciones principales:

1. Percepción o sensación producida por un alimento introducido en la boca.
2. Atributo del alimento percibido o conjunto de sus características.

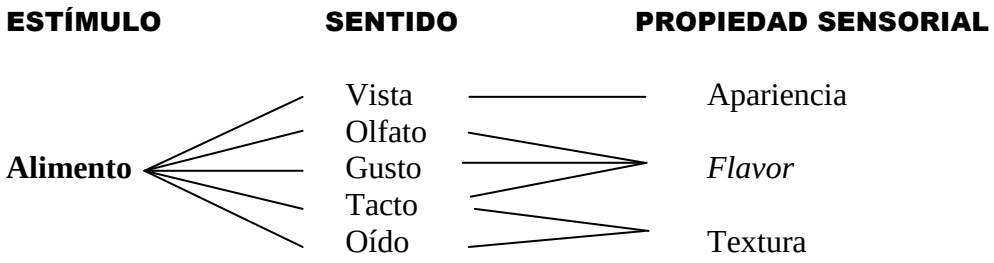


**Figura 2.** Relaciones entre los sentidos y las percepciones (Fisher y Scott, 1997)

Los consumidores consideran el *flavor*, junto con la apariencia y la textura, una de las tres propiedades sensoriales decisivas en la elección (figura 2). Si el *flavor* se refiere a los elementos químicos responsables de la estimulación o si se refiere a la recepción de la estimulación biológica en sí, no importa especialmente al consumidor. El *flavor* se percibe principalmente por los receptores aromáticos de la nariz y los receptores de sabor de la lengua (las papilas gustativas). A estas percepciones hay que sumar otras sensaciones producidas en la lengua al ingerir el alimento (respuesta trigeminal) como son su temperatura, astringencia, etc. Generalmente se admite que las sensaciones del gusto son cuatro (salado, dulce, ácido y amargo); sin embargo, algunos autores incluyen una quinta denominada "umami" que podríamos entender en castellano como "sabrosidad" y que puede ser representada por el *flavor* del glutamato (Fisher y Scott, 1997).

La interacción física entre los compuestos volátiles y el lugar de recepción ocurre en los conductos nasales. Esas moléculas alcanzan las terminaciones nerviosas olfativas, se unen de manera análoga a las asociaciones enzima-substrato tanto a través del conducto nasal como por vía oral, y desencadenan reacciones odoríferas. El sistema olfativo es el más sensible de todos los sentidos (Baldwin *et al*., 2000). Sin embargo, como contrapartida a esta extremada sensibilidad presenta la facilidad con que puede alcanzarse cierta fatiga, lo que debe ser una defensa ante posibles daños nerviosos (DeRovira, 1997).

El olor es la percepción de las sustancias volátiles liberadas desde los alimentos de forma espontánea y a través del ambiente, tras ser reconocidas

por los receptores localizados en el interior de las células de la mucosa pituitaria que recubre el interior de la nariz. Captado el estímulo, la señal es enviada al cerebro para ser interpretada (Lavilla, 1998).

El aroma, por su parte, es la percepción de las sustancias denominadas en este caso "aromáticas" después de la introducción de los alimentos en la boca y su masticación. En este proceso los componentes aromáticos se disuelven en la mucosa del paladar y de la faringe. La temperatura bucal, la ruptura de las células de los alimentos con la masticación y la reducción de la viscosidad al efectuarse la salivación, hacen que los volátiles se liberen (Land, 1994; Taylor y Linford, 1994) para llegar por vía retronasal, a través de la trompa de Eustaquio, a los receptores olfativos de la nariz (Lavilla, 1998).

El olor y aroma característicos de cualquier fruta se deben a la existencia de compuestos volátiles (en piel, pulpa y mucílago, en el caso del tomate) (Buttery, 1981; Fellman *et al.*, 2003) formando una compleja mezcla de la que es importante distinguir sus componentes así como conocer el impacto que se les debe atribuir, y su naturaleza (momento y reacciones por las que son sintetizados y su posterior degradación en otros compuestos).

No todas las sustancias odorantes son iguales. Los odorantes primarios definen, cada uno, un olor individual y pueden entonces combinarse para dar lugar a aromas. Cada uno se une a un único receptor del bulbo olfatorio. Son como las letras del abecedario. Los odorantes secundarios son como sílabas y pueden unirse a varios receptores, lo que da lugar a que un mismo compuesto sea descrito de diversas formas (DeRovira, 1997). Además en el *flavor* existen las conocidas como notas principales que suelen ser muy identificables en los alimentos, dado su carácter polar y termolábil, su peso molecular relativamente bajo, generalmente, y su elevada volatilidad. Las notas secundarias, sin embargo, suelen ser de bajo peso molecular, más termoestables y apolares y tener un impacto mucho más sutil en el sabor (Baldwin *et al*., 2000). En el tomate se han encontrado e identificado más de 400 compuestos volátiles y, sin embargo, con sólo 10 de ellos se podría imitar de un modo bastante fiel el aroma del tomate fresco (Buttery, 1993).

Un concepto de utilidad al tratar con aromas es el de Unidad Odorífera ($U_o$) que es la razón entre la concentración a la que está presente un compuesto en un alimento y su umbral de detección, normalmente en agua

(generalmente en alimentos con elevado contenido en agua se asume que los volátiles se comportan como en disolución acuosa). Si el valor de esta unidad $U_o$ es mayor de 1, el compuesto en cuestión contribuye en la percepción global pero si fuese inferior a 1 probablemente no contribuiría al aroma (Buttery, 1993).

Olfato y gusto interactúan para proporcionar una visión integrada (Voirol y Daget, 1987). Según Tieman *et al.* (2006), el *flavor* del tomate es el resultado de la interacción entre azúcares, ácidos y un conjunto de aproximadamente 30 compuestos volátiles. Marcadores moleculares ligados a estos compuestos podrían ser de gran utilidad en programas de mejora con el objetivo de conseguir frutos de mejor *flavor*. A largo plazo, los genes responsables de controlar los niveles de estos elementos serán importantes herramientas para comprender las complejas interacciones que finalmente se integran para dar lugar al *flavor* del tomate.

#### 1.3.1.2. Calidad nutricional

El tomate no es sólo un componente importante de la dieta mediterránea sino también de otras dietas. Existen evidencias de que su consumo regular reduce la incidencia de enfermedades degenerativas crónicas como algunos tipos de cáncer (Giovannucci, 1999) y otras enfermedades cardiovasculares (Pandey *et al.*, 1995). Estos efectos beneficiosos suelen ser atribuidos a los carotenoides, que parecen disminuir el riesgo de ciertos tipos de cáncer, arterioesclerosis y la formación de cataratas (Sandstrom *et al*, 1994; Weisburguer, 1998; Frusciante *et al.*, 2007).

La calidad nutritiva de los productos vegetales depende de la cantidad y calidad de los macronutrientes (proteínas, carbohidratos y lípidos) y de los micronutrientes (vitaminas, elementos minerales, ácidos grasos y aminoácidos esenciales) que proporcionan. Hoy día se habla de compuestos “bioactivos” en referencia a compuestos de origen vegetal con acción beneficiosa para la salud (Cámara, 2006). El valor nutritivo del tomate no es muy elevado (tabla 1). Como media, el contenido en materia seca de un fruto fresco maduro oscila entre 5.0 y 7.5% (Petro-Turza, 1987).

| Constituyente | % |
|---|---|
| Fructosa | 25 |
| Glucosa | 22 |
| Sacarosa | 1 |
| Ácido cítrico | 9 |
| Ácido málico | 4 |
| Proteínas | 8 |
| Ácido amino dicarboxílico | 2 |
| Substancias pécticas | 7 |
| Celulosa | 6 |
| Hemicelulosa | 4 |
| Minerales | 8 |
| Lípidos | 2 |
| Ácido ascórbico | 0.5 |
| Pigmentos | 0.4 |
| Otros aminoácidos, vitaminas y polifenoles | 1 |
| Volátiles | 0.1 |

**Tabla 1.** Composición del contenido de la materia seca de tomate (Petro-Turza, 1987).

Así destacan cuantitativamente los azúcares con prácticamente el 50% de la materia seca, los ácidos orgánicos son el 15%, los aminoácidos libres que suponen entre un 2 y un 2.5% y, los minerales el 8% de la materia seca (Yilmaz, 2000). De los minerales, el potasio y los fosfatos son los mayoritarios. Los minerales tienen un efecto en el pH, la acidez valorable y poseen capacidad amortiguadora, de esta forma influyen en el sabor de los tomates (Petro-Turza, 1987).

El efecto beneficioso de los tomates se atribuye generalmente a los carotenoides. En el tomate están presentes principalmente dos de ellos, el licopeno (figura 3) que es el de mayor contenido (entre un 80 y un 90%) y el β-caroteno (que supone el 7 al 10% de los carotenoides totales). El tomate representa con mucho la mayor fuente de licopeno (compuesto con fuerte capacidad antioxidante), mientras que otras fuentes alimentarias contribuyen al

consumo diario de β-caroteno, cuyo principal interés es tener actividad provitamina A. La importancia del licopeno radica tanto en que su actividad antioxidante es el doble que la del β-caroteno como por formar parte de otros mecanismos hormonales y metabólicos como el hecho de intervenir en la comunicación intercelular y modular los mecanismos inmunológicos (Martín-Moreno y Gorgojo, 2002; Rao y Agarwar, 2000; Cámara, 2006).

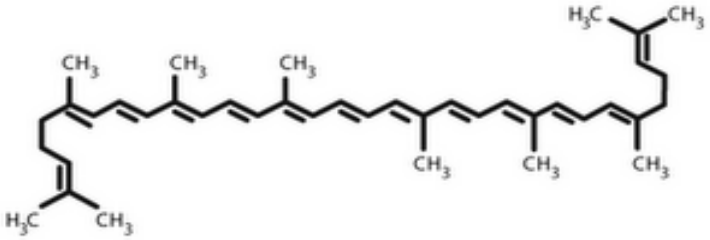

**Figura 3.** Estructura química del licopeno ($C_{40}H_{56}$) presente en los vegetales (Periago *et al.*, 2001).

No obstante el tomate contiene otras moléculas potencialmente saludables como el ácido ascórbico, la vitamina E y compuestos fenólicos, particularmente flavonoides. La importancia de los tomates para el consumo diario de vitamina C es variable de acuerdo con los hábitos de consumo, pero se considera una buena fuente de vitaminas C y A. Es más, se sitúa en el puesto 13 del ranking de frutas y hortalizas como fuente de los aportes de vitamina C (Abdullahi y Choji, 2009). Sin embargo, la contribución del tomate a la ingesta total de vitamina E es puramente marginal. Los polifenoles del tomate, sobre todo los ácidos fenólicos, están libres, en forma soluble y también están presentes de forma insoluble unidos a la fibra (Frusciante *et al.*, 2007). Los compuestos polifenólicos están adquiriendo cada vez mayor protagonismo como agentes bioactivos. Son un amplio grupo de sustancias que incluyen los flavonoles, catequinas, antocianinas, y que pueden encontrarse en los vegetales aisladamente o unidos a azúcares (glicósidos), aunque no todos tienen importancia nutricional (Cámara, 2006).

El tomate tiene además flavonoides, en concreto rutina y naringina, y hay informes que indican que pueden jugar un papel de sustancial importancia en los beneficios que aporta el consumo de tomate a la salud gracias a su elevado poder antioxidante. Finalmente, el tomate es también una fuente relevante de fibras, constituidas por pectinas, hemicelulosa y celulosa y presenta además un contenido importante de ácidos orgánicos, fundamentalmente ácido cítrico (Frusciante *et al.* 2007).

Hasta hace poco la calidad nutricional no había sido un factor determinante en los programas de mejora desarrollados por las casas comerciales. Aunque eso no impide que también se hayan hecho intentos de abordar posibles enfoques de mejora, por ejemplo mediante la sobreexpresión de la enzima fitoeno sintasa que logró incrementar los niveles de licopeno (Fraser *et al.*, 2002). Otro ejemplo, al expresar dos factores de transcripción procedentes del maíz, se ha mejorado el contenido en la pulpa del flavonol campeferol (Bovy *et al.*, 2002). Con la introducción del gen que expresa la Δ6 desaturasa Cook *et al.* (2002) consiguieron un aumento en los porcentajes de ácido γ-linolénico $_{(18:3)}$ y de ácido octadecatetraenoico junto a una inesperada reducción de ácido linoleico $_{(18:2)}$ (Cámara, 2006).

1.3.1.3. Calidad poscosecha

Entendemos por un buen comportamiento poscosecha de frutas y hortalizas la capacidad para retardar al máximo posible la disminución de su calidad general a lo largo del período de almacenamiento. Esto es difícil debido a la naturaleza perecedera de los alimentos. De forma natural las frutas y hortalizas, una vez cosechadas, comienzan a sufrir una serie procesos de degradación. Así, por ejemplo, se dan pérdidas de peso puesto que se pierde humedad, se produce un ablandamiento de los tejidos como respuesta de la actividad de los enzimas poligalacturonasa (PG) y pectinmetilesterasa (PME) en las paredes celulares, el perfil aromático sufre también alteraciones importantes, etc.

Desde el punto de vista genético ha habido intentos de superar esta cadena de alteraciones, como el desarrollo del tomate FlavorSavr$^{TM}$, que fue

modificado para retrasar su maduración, y por consiguiente ampliar su período de conservación en las cadenas de suministro (Martineau, 2001) y que se distribuyó en el mercado de productos frescos entre 1994 y 1996. Se introdujo un gen antisentido PG para inhibir la expresión del enzima y permitir así cosechar más tarde (Cámara, 2006). La regulación de los enzimas digestores de la pared celular (PG y PME) en frutos transformados con genes antisentido inhibió su actividad a menos del 1% del nivel habitual aumentando así la vida útil del producto o *shelf-life* e incrementando también su resistencia a enfermedades y el contenido en sólidos (Hobson y Grierson, 1993; Kramer *et al.*, 1992; Schuch *et al.*, 1991). No obstante este tomate fue retirado del mercado hacia 1996. Su fracaso se debió a que se escogió una variedad poco adecuada. La propia compañía Calgene reconoció que no tenía un rendimiento aceptable ni las resistencias adecuadas. Así mismo su calidad organoléptica también debía ser mejorada (Nature, 1996).

Otras aproximaciones para mejorar este aspecto de la conservación de los frutos se han basado en el control de las reacciones que transcurren en la etapa de poscosecha relacionadas con el *flavor*. Así pues se han clonado genes (*tomloxA* y *tomloxB*) que intervienen en la actividad del enzima lipoxigenasa (LOX) que interviene en la conversión del ácido linoleico $_{(18:2)}$ en hexanol, los conocidos mutantes *nonripening* (*nor*) y *never-ripe* (*Nr*). Otros dos genes (*adh1* y *adh2*) fueron identificados en tomate pudiendo con su manipulación incidir en el funcionamiento del enzima alcohol deshidrogenasa (ADH) que transforma el hexanal y el *cis*-3-hexenal en hexanol y *cis*-3-hexenol, respectivamente. Además cabe apuntar que la firmeza y capacidad *shelf-life*, sin embargo, a menudo son mayores en los híbridos (Baldwin *et al.*, 2000).

La calidad poscosecha desde el punto de vista de la comercialización también es abordada a menudo, pues como el consumidor de hoy día busca alternativas de alimentación saludable pero su estilo de vida en muchos casos está marcado por falta de tiempo para preparar y comer los alimentos, se prefiere productos alternativos nutritivos, sabrosos, variados y fáciles de preparar. En este sentido, los vegetales mínimamente procesados, también conocidos como cuarta gama o listos para consumir, están dirigidos a satisfacer esta demanda actual.

Las tecnologías tradicionalmente empleadas en la conservación de tomate son la **refrigeración** (como requisito indispensable tanto en las etapas de producción, como de distribución, almacenamiento y comercialización) y el **envasado en atmósfera modificada.** Aún con el envasado y la refrigeración la vida útil de la mayoría de frutas y hortalizas frescas no va más allá de unos pocos días. La investigación para comercializar frutos prolongando al máximo la calidad poscosecha es una línea en continuo desarrollo. La introducción de paladio, incorporado a carbón activado extrusionado, en envases herméticos, reduce la acumulación de etileno permitiendo que el fruto mantenga un aspecto externo (color y firmeza) más próximo a los del fruto recién recolectado (Bailén *et al.*, 2006). Otros ensayos han estudiado el efecto del 1-metilciclopropeno (1-MCP) sobre el comportamiento poscosecha del tomate, un bloqueante de los receptores de etileno del fruto, que ya ha sido probado con éxito en diversas frutas y hortalizas (Valverde *et al.*, 2003). Tratamientos como la inmersión en disoluciones de sales de calcio para conservar la firmeza del producto, agentes antioxidantes para controlar los cambios de color y el uso de sustancias antimicrobianas para controlar el crecimiento de microorganismos indeseables son empleados frecuentemente para ayudar a conservar la calidad de las frutas y hortalizas (Rojas-Grau, 2006; Garcia y Barret, 2002).

El uso de recubrimientos y tecnologías de envasado activo están encontrando creciente éxito para algunos productos. Se dispone en la actualidad de una amplia gama de envases de diferentes materiales con características adecuadas para cubrir la diversidad de demandas específicas que plantean las sucesivas fases de la manipulación, transporte, almacenamiento y comercialización de frutas y hortalizas.

### 1.3.2. Técnicas de determinación

Muchos atributos de la calidad carecen o han carecido durante mucho tiempo de los métodos adecuados de evaluación (Llácer *et al.*, 2006). A continuación vamos a analizar las posibilidades que se presentan para determinar los aspectos de la calidad que nos interesan.

1.3.2.1. Calidad organoléptica o sensorial

Existen determinaciones analíticas sencillas para medir algunos de los atributos de la calidad organoléptica como la textura, el contenido en sólidos solubles, la acidez valorable, el color… (Valcarcel, 2009).

La textura es una característica que resulta de la percepción de la pulpa del fruto en la boca, de la presencia del gel contenido en los lóculos del fruto y del grosor o la elasticidad de la piel. Otros criterios utilizados para caracterizar la textura sensorialmente son la harinosidad de la pulpa y la presencia de zonas fibrosas (Valcarcel, 2009). También es posible determinar la firmeza o dureza de los frutos con medidas objetivas, tal es el caso del test Magness-Taylor que hace referencia a la resistencia de la piel y la firmeza puesto que implica pruebas de compresión y corte o bien del test de compresión que da una idea de la dureza o firmeza del alimento al someterlo a determinada fuerza de compresión.

El color puede quedar caracterizado con el sencillo manejo de cartas de color, pero resulta igualmente sencillo y es mucho más preciso el uso de colorímetros que utilizan la escala de color de la CIE *L*a*b** (1976) donde la variable *L* hace referencia a la luminosidad, el parámetro "*a*" es un índice que va del verde al rojo, y el "*b*" del azul al amarillo. Con estos parámetros pueden posteriormente calcularse otros índices aplicados al color (ángulo de Hue, etc.).

Los sólidos solubles se determinan de forma sencilla con un refractómetro y la acidez valorable se obtiene mediante una reacción de valoración en la que empleamos NaOH. Sin embargo, los componentes de la calidad interna como el sabor, el aroma, el *flavor*… son de determinación más compleja.

Para evaluar el sabor global se recurre a paneles de cata con jueces entrenados. La evolución del análisis sensorial de los alimentos se produce en 3 áreas:

1) mediante la normalización de los ensayos y la acreditación de laboratorios sensoriales (existen 26 normas ISO bajo el epígrafe 67.240 disponibles en www.iso.org y 23 normas UNE en www.aenor.es ),

2) mediante el desarrollo de sistemas informáticos específicos para la captura y análisis de datos (son muchos los programas comercialmente disponibles como el Computense *five*, FIZZ, Sims o Tastel),
3) mediante la mejora de la metodología sensorial, con la puesta a punto de nuevos métodos de evaluación y desarrollo y la adaptación de nuevas técnicas estadísticas para el tratamiento de los datos sensoriales (Casañas y Costel, 2006). A la hora de formar un panel de catadores de tomate, el objetivo es seleccionar entre los candidatos a los futuros jueces, mediante un entrenamiento específico. En el reclutamiento, hay que realizar una selección previa para descartar candidatos no aptos para llevar a cabo evaluaciones sensoriales. Además, se tienen en cuenta otros factores como la posibilidad de sufrir reacciones alérgicas, dificultad de comunicar las percepciones recibidas durante la cata, el interés o la motivación que les mueven a participar (Ruiz *et al.*, 2005).

La búsqueda de modelos predictivos que permitan aproximarnos al sabor a partir de sencillas determinaciones en los frutos es una de las líneas de investigación que más interés despiertan. Encajan en esta dirección estudios realizados para encontrar correlaciones entre ciertas determinaciones analíticas y entre dichas medidas y los descriptores empleados en las evaluaciones sensoriales, como el del cis-3-hexenal que se relaciona con el descriptor “típico de tomate” (Abegaz *et al.*, 2004) o bien los que, en función de los resultados de determinadas variables incluirían el fruto dentro de un tipo varietal u otro (García-Aliaga, 2008).

#### 1.3.2.1.1. Técnicas empleadas en el estudio de los compuestos volátiles

En cuanto al estudio de los aromas es fundamental la importancia de la técnica de extracción que se emplea. Encontramos en la literatura un rango de valores para los compuestos volátiles presentes en tomate muy amplio, y esto en parte es debido a la falta de estandarización en las técnicas de extracción

de la fase volátil. En un principio los procedimientos de extracción empleados se basaban en destilación por vapor y la extracción con disolventes (Teranishi y Kint, 1993) pero de esta forma se puede alterar el perfil de la muestra, tanto cuantitativa como cualitativamente (Schamp y Dirinck, 1982). También ha sido utilizado el método del purga-trampa en que se hace circular a través de la muestra una corriente de gas inerte y así los volátiles emitidos van siendo atrapados en una trampa adsorbente que será después rápidamente calentada para liberar los compuestos. Este método de análisis ha sido empleado por nuestro equipo para realizar análisis cuantitativos de los volátiles en accesiones de variedades tradicionales de tomate de los tipos Muchamiel y De la Pera, incluyendo también un híbrido comercial como referencia (Ruiz *et al.*, 2005). En ese trabajo se detectaron diferencias en el contenido de algunos compuestos volátiles que mayor impacto parecen tener en el tomate, tanto a nivel de tipo varietal como entre accesiones del mismo tipo, así como para la aceptación general. Estos resultados pusieron de manifiesto la complejidad de la cuantificación de los compuestos volátiles, así como su importancia en la percepción de los distintos atributos de calidad, lo que nos animó a estudiar otros compuestos volátiles. Así, en Alonso *et al.* (2009a), se estudiaron un mayor número de compuestos en accesiones del tipo “Muchamiel” y “De la Pera”. Los mejores resultados de las evaluaciones sensoriales, así como un mayor contenido de muchos de los compuestos volátiles presentes, correspondieron a los tipos tradicionales. El análisis discriminante utilizado permitió distinguir los tipos varietales estudiados (“De la Pera”, “Muchamiel” e híbrido) utilizando solamente 6 de los 10 compuestos volátiles. Este resultado es interesante porque podría permitir una reducción del número de componentes analizados si queremos conocer el tipo varietal al que pertenecen los frutos analizados.

Sin embargo los métodos de espacio de cabeza estático parece que reflejan más fielmente el verdadero perfil aromático del tomate. Dentro de este grupo tenemos las trampas frías de los volátiles en el espacio estático de cabeza (Teranishi y Kint, 1993) en las que las muestras se pueden concentrar sin los inconvenientes que supone el calentamiento. Otra técnica rápida es la microextracción en fase sólida que se basa en la interacción de los volátiles con un material de gran afinidad con los volátiles, que los retiene. Nuestro

grupo ha realizado un estudio comparativo entre tres técnicas de extracción de compuestos volátiles del tomate empleando una línea de mejora de nuestro programa y la tradicional original (Alonso *et al.*, 2009b). De los métodos de extracción estudiados los resultados de la extracción y destilación simultánea (SDE) y la hidrodestilación (HD) fueron los que mejor se correlacionaron con el aroma de tomate fresco. Sin embargo, la microextracción de fase sólida (SPME) podría ser una herramienta útil para lograr valores reales del olor a tomate fresco. Los datos obtenidos con la SPME mostraron correlación positiva con el olor obtenido en la calificación sensorial realizada, mientras que los otros tipos de extracción, la HD y la SDE, se correlacionaron positivamente con el aroma evaluado.

Una vez se ha obtenido la fase volátil se analiza con cromatografía de gases (GC) o sistemas combinados de cromatografía de gases y espectrometría de masas (GC/MS) para identificar y cuantificar los compuestos. En este tipo de análisis se incorporan patrones internos a las muestras. Como los compuestos volátiles continúan su evolución en el extracto resultante, la desnaturalización o inhibición enzimática son necesarias. Entre los compuestos inhibidores más usados están el cloruro de sodio y, más eficaz todavía, el cloruro cálcico (Buttery, 1993).

El análisis de aromas tiene ciertas particularidades, por ejemplo, suele tratarse de mezclas heterogéneas, en concentraciones muy reducidas y alterables. Además la percepción de los aromas se ve influenciada por otros aspectos (contenido en azúcares, ácidos, taninos, etc.) por lo que resulta necesario comparar los resultados cromatográficos con los resultados obtenidos en análisis sensorial (Maqueira y Puchades, 2006).

#### 1.3.2.2. Calidad nutricional

El método de determinación depende del componente a analizar. Así por ejemplo calcio, magnesio, hierro, cobre, manganeso y zinc se determinan por espectrometría de absorción atómica, pero para potasio y sodio es habitual recurrir a la fotometría de emisión. Para cuantificar el contenido de carotenoides, compuestos fenólicos y vitaminas se recurre a la técnica

cromatográfica conocida como HPLC (High Performance Liquid Chromatography). El licopeno es el carotenoide mayoritario del tomate. Para comprobar si la introducción de los genes de resistencia en las variedades tradicionales ha tenido un efecto (negativo o positivo) en su contenido, se ha realizado la puesta a punto de su cuantificación en varias líneas de mejora del programa, así como en las variedades tradicionales originales y en un híbrido comercial. Los resultados de este estudio se recogen en un trabajo que se enviará al XVIII Congreso Internacional de Horticultura (ISHS) de Lisboa 2010.

#### 1.3.2.3. Calidad poscosecha

En primer lugar, se definen los parámetros que se pretende estudiar durante la conservación de los frutos. Encontramos en este punto como los más interesantes la textura del fruto, el color, la evolución de la respiración y de la producción de etileno, la acidez valorable, el contenido en sólidos solubles, las alteraciones a nivel del contenido en compuestos volátiles y su evaluación sensorial global. Las técnicas de evaluación de los componentes de la calidad en estudios de poscosecha, corresponden a las que se suelen utilizar para cada uno de los parámetros de calidad seleccionados.

## 1.4. Comparación de caracteres de calidad entre variedades tradicionales y líneas de mejora

La introducción de resistencias a enfermedades y patógenos utilizando donantes de baja calidad sensorial ha sido una fuente de disminución en las características organolépticas de los cultivares (Casañas y Costell, 2006). Se ha observado en algunas condiciones un efecto negativo de los genes de resistencia introducidos sobre caracteres como la producción, contenido en azúcares, etc. (Tanksley *et al.*, 1998; Brown, 2002; Lewis *et al.*, 2007). Este hecho puede ser debido al efecto desfavorable de los genes de resistencia o a otros genes que van en el mismo fragmento de cromosoma introducido. Esta es la razón por la que se debe comparar la calidad de las líneas de mejora con las variedades tradicionales de las que deriva. En nuestro grupo de trabajo se

han realizado varios estudios al respecto y se ha comprobado el efecto desfavorable del gen *Ty-1* para algunos caracteres agronómicos (producción y cuajado) y para determinados parámetros de calidad organoléptica (acidez valorable) (García-Martínez *et al.*, 2008).

### 1.4.1. Estudio del comportamiento poscosecha

Debido a las características del tomate, el método de conservación más empleado es el almacenamiento refrigerado a una temperatura comprendida entre 8 y 10 ºC y una humedad relativa cercana al 90%. En estas condiciones el período de conservación en un estado apto para el consumo oscila alrededor de 15 días para las variedades tradicionales y entre 25 y 30 días en el caso de los híbridos comerciales. En este aspecto es fundamental el estado de maduración en el momento de la recolección, así como la ausencia de lesiones o daños en los frutos.

Los trabajos de poscosecha incluyen también el análisis del período *shelf-life* en que los frutos se mantienen durante un corto período de tiempo en condiciones ambientales, como el consumidor los puede tener en su propio hogar unos días antes de ser consumidos. En este caso igualmente se analizan los cambios en los parámetros ya definidos como indicadores de la evaluación de la madurez del fruto.

Tras comprobar el efecto negativo de los genes de resistencia introducidos sobre algunos caracteres agronómicos y de calidad, se realizó un estudio para comprobar si estos genes también tenían efecto en distintos parámetros de calidad durante la conservación frigorífica de una línea de mejora Muchamiel del programa y la variedad tradicional original, cultivadas en condiciones de agricultura ecológica. Los resultados completos se encuentran recogidos en Alonso *et al.* (en prensa). La línea de mejora mantuvo valores más elevados durante la conservación para la firmeza y dureza de los frutos, mientras que no hubo diferencias significativas entre líneas para el resto de los parámetros de calidad estudiados. Nuestros resultados indican que es posible introducir resistencia genética a virosis sin alterar la calidad organoléptica de la variedad original.

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# RESULTADOS DE UN PROGRAMA DE MEJORA GENÉTICA PARA LA INCORPORACIÓN DE RESISTENCIA A VIROSIS EN VARIEDADES TRADICIONALES DE TOMATE

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**RESUMEN**

En 1998 se inició un programa de mejora destinado a posibilitar la recuperación del cultivo de algunas variedades tradicionales de tomate del sureste de español. El cultivo de dichas variedades comenzó a verse desplazado a partir del comienzo de la agricultura intensiva, hasta desembocar en la situación actual, en la que prácticamente se ha abandonado por ser inviable dada la susceptibilidad que presentan a las principales virosis que afectan al tomate. Sin embargo, hoy en día se constata un descontento creciente por parte de los consumidores, que consideran que el tomate presente en nuestros mercados carece del sabor típico de las variedades tradicionales. El objetivo del programa de mejora era la incorporación de resistencias genéticas al virus del mosaico del tomate (ToMV), al virus de la cuchara (TYLCV) y al virus del bronceado (TSWV) en algunas variedades de tomate tradicionales.

## INTRODUCCIÓN

El tomate es uno de los cultivos más importantes en España en superficie (63.000 ha), en producción (3.947.300 Tm) y especialmente en valor económico (1.937.743.000 euros), donde sólo es superado por el olivo y el viñedo, cultivos cuya superficie es claramente superior (Anuario de Estadística Agroalimentaria, 2006; Anuario de Producción FAO, 2006). Aunque se cultiva en toda España, es en el sureste peninsular y en Canarias donde se concentra la producción para tomate en fresco.

El tomate es un cultivo que presenta una amplia diversidad en cuanto a forma, color, sabor, textura y tamaño del fruto (Figura 1), entre otros aspectos, lo cual le confiere gran atractivo en los mercados. También hay que considerar que se trata de un producto muy versátil ya que puede destinarse a consumo en fresco en ensaladas, como fruta, o bien procesado. Y aunque no se trata de una importante fuente de vitaminas, continúan apareciendo estudios que atribuyen al licopeno (el carotenoide mayoritario en el tomate y responsable de su color rojo) propiedades anticancerígenas y con efectos cardiovasculares positivos (Bowen *et al.*, 2002; Ansari y Gupta, 2004; Frusciante *et al.*, 2007), lo cual supone un importante valor añadido para este producto. Todo ello unido a que se trata de un cultivo tradicional, y muy ligado a la tan reconocida "dieta mediterránea", lo convierten en un producto enormemente estimado.

No obstante existe una queja muy frecuente por parte de los consumidores que denuncian la pérdida del "sabor característico de tomate" en las variedades actuales.

La susceptibilidad a virosis es el principal motivo por el cual cultivar variedades tradicionales resulta inviable en muchas zonas. La finalidad de este programa es obtener variedades de calidad organoléptica lo más similar posible a la de tipos tradicionales (tipo Muchamiel y tipo De la pera) pero con resistencia genética a tres de las virosis más importantes. Como recomienda la FAO (Organización para la Agricultura y la Alimentación) y el IPGRI (Instituto Internacional de Recursos Fitogenéticos) se ha implicado a agricultores de la zona en el programa de mejora. Existen otras virosis y alteraciones importantes en este cultivo pero hasta que se disponga de fuentes de resistencia efectivas no se podrá plantear su introducción.

Las variedades tradicionales de tomate presentan una forma característica, existe diversidad genética entre los distintos tipos (Ruiz *et al.*, 2005a; García-Martínez *et al.*, 2006) y también elevada variabilidad en cuanto a parámetros tales como color, textura, aceptación general, contenido en micronutrientes, aromas, ácidos y azúcares, producción de etileno y respiración (Ruiz *et al.*, 2005b; Ruiz *et al.*, 2005c; Carbonell-Barrachina *et al.*, 2006; Ruiz *et al.*, 2006; Alonso *et al.*, 2008). Estas variedades tradicionales tan particulares y apreciadas deben ser preservadas y su valor justifica el programa de mejora para introducir algunos genes de resistencia y permitir así su continuidad en cultivo. Sin embargo, el objetivo final de este programa no es desarrollar una sola variedad resistente, sino una colección de genotipos resistentes para cada tipo tradicional, posibilitando así mantener cierto grado de diversidad.

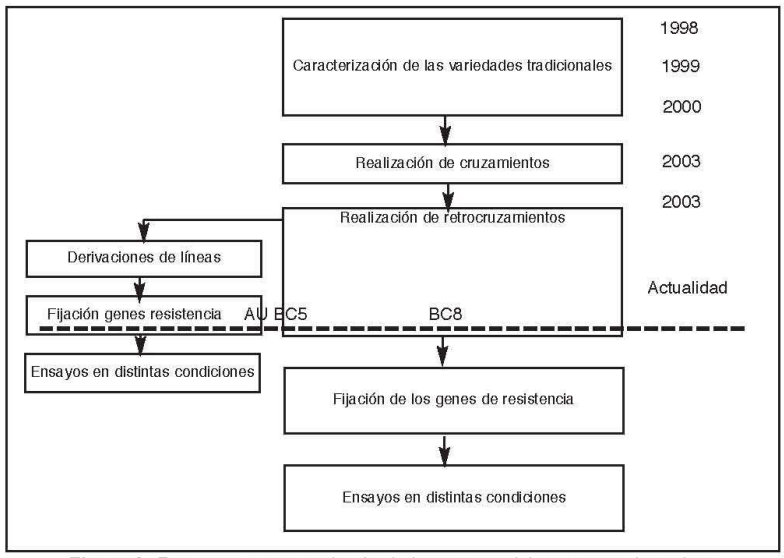


**Figura 3:** Resumen esquematizado de las etapas del programa de mejora.
**BC**: retrocruce; **AU**: autofecundación.

## MATERIAL Y MÉTODOS

El programa de mejora comenzó con la caracterización de una colección de variedades tradicionales de tomate de los tipos "De la pera", "Muchamiel" y "Moruno" (Figura 2), realizada entre 1998 y 2000 en distintos ciclos y bajo condiciones diversas. De esta forma se eligieron las mejores accesiones y se procedió a realizar cruzamientos con un híbrido comercial resistente al virus del bronceado y del mosaico, y tolerante al virus de la cuchara.

De partida se eligieron 5 variedades tradicionales tipo Muchamiel, otras 5 del tipo "De la pera", 6 "Morunos" y 4 de otros tipos. Los primeros cruces se realizaron durante el ciclo de otoño-primavera de 2003. Con las plantas procedentes de las semillas de estos frutos, se empezó el proceso de retrocruzamiento (Figura 3) con las variedades tradicionales, para recuperar las características de éstas. En función de las diferencias genéticas entre los parentales (las variedades tradicionales y el híbrido comercial, en nuestro caso) el número de retrocruces necesario se estimó entre 5 y 10. En cada ciclo se debe discriminar las plantas que portan los tres genes de resistencia, que se retrocruzan, de las que no los portan, que se desechan. En este caso, solamente una de cada 8 plantas tendrá todos los genes de resistencia. El empleo de marcadores moleculares ligados a estos genes de resistencia permite hacer esta selección en estado de plántula. La selección precoz permite transplantar, y cultivar, solamente los individuos con todos los genes de resistencia, lo que se traduce en un ahorro de espacio, mano de obra y recursos. No obstante, aún empleando estas técnicas sigue siendo necesaria una confirmación fenotípica de la resistencia/tolerancia de las plantas, para evitar cometer errores en la selección. Estos errores pueden ser más frecuentes cuando se trata de tolerancia a la virosis como es el caso del virus de la cuchara, ya que en determinadas condiciones (niveles elevados de inóculo, condiciones ambientales estresantes) la tolerancia puede ser superada. Por lo tanto, es preferible utilizar la selección genotípica combinada con la fenotípica, que ha mostrado ser la estrategia óptima en diversos estudios (García-García, P., 2004).

Los marcadores utilizados inicialmente fueron descritos previamente por otros autores. Al comprobar que en nuestro material uno de los marcadores se comportaba como dominante (incapaz de distinguir los individuos homocigotos resistentes de los heterocigotos) se llevó a cabo el desarrollo de nuevos marcadores codominantes, que sí distingan los tres genotipos, y que se están utilizando actualmente en el programa de mejora (García-Martínez *et al.* 2003).

**Figura 1:** Frutos de distintas variedades de tomate, tanto tradicionales como híbridos comerciales.

**Figura 2**: Frutos de las variedades tradicionales **Muchamiel** y **De la pera**.

▲**Figura 4a**

◀ **Figura 4b**

**Figuras 4a y 4b.** Plantas pertenecientes a retrocruces de sexta generación de los tipos varietales **De la pera** y **Muchamiel** cultivadas en invernadero y al aire libre

**Figura 5** ▶
Ensayo llevado a cabo por un agricultor colaborador en el programa de mejora.

## RESULTADOS Y DISCUSIÓN

El programa de mejora ha ido avanzando a un ritmo de dos retrocruces al año, gracias a la realización de ciclos de cultivo bajo invernaderos. Los ciclos realizados son de invierno-primavera (transplantando en Febrero-Marzo y recogiendo los frutos en Junio-Julio) y el de verano-otoño (transplantando en Agosto-Septiembre y recogiendo en Noviembre-Enero), disponiendo de muy escaso tiempo entre los dos ciclos.

Se realiza una selección precoz en semillero utilizando los tres marcadores moleculares. Durante todo el ciclo de la planta se comprueba que ésta no tenga síntomas de ninguna de las virosis. Entre las plantas resistentes según los marcadores y que no manifiesten síntomas, se realiza una selección para caracteres agronómicos deseables (cuajado, producción, susceptibilidad a podredumbre apical, etc..) y de conformidad a tipo, para recoger los frutos de retrocruce y sembrar sus semillas en el siguiente ciclo. La resistencia conferida por los genes introducidos se ha mostrado efectiva hasta el momento.

Actualmente se dispone de varias familias de retrocruces de novena generación, los cuales a su vez se están retrocruzando. El grado de recuperación de las características morfológicas de estos retrocruces se puede calificar como aceptable (Figuras 4a y 4b).

A partir del quinto retrocruzamiento se derivaron líneas que se ofrecieron a distintos agricultores de la Vega Baja del Segura para su cultivo. Esta derivación se realizó cuando se estimó que las líneas tenían un nivel suficiente de aceptación, tanto por parte del agricultor como por parte del consumidor. El resultado obtenido se considera muy positivo, aunque las líneas cultivadas por estos agricultores no tengan fijados los tres genes de resistencia ni hayan recuperado todas las características morfológicas y organolépticas de las variedades tradicionales. Los frutos de estas líneas se han estado vendiendo en la Lonja de Orihuela a precios considerablemente superiores a los de los híbridos comerciales.

La participación de los agricultores en la mejora de las variedades es muy importante, ya que permite llevar a cabo ensayos de mayores dimensiones y en condiciones de cultivo real, y permite seleccionar en distintos ambientes destino el material que va generando el programa de mejora. Además, esta estrategia contribuye a hacer partícipe a los agricultores del programa de mejora, aprovechando sus excelentes conocimientos sobre las variedades y técnicas de cultivo, y contribuye a que conozcan y valoren el esfuerzo que supone dicho programa (Figura 5). Así mismo, el cultivo de un gran número de plantas en campos de los agricultores permite obtener la cantidad de frutos de cada una de las líneas, en condiciones homogéneas y en una sola recolección, necesarios para la evaluación de la calidad organoléptica y capacidad de conservación de las líneas del programa de mejora. Esta es una fase fundamental de todo programa, y se realiza en las últimas etapas del mismo para comprobar si, además de las nuevas características introducidas, las plantas mejoradas han recuperado todas las propiedades de las variedades tradicionales. En esta fase final se ha aumentado fuertemente el número de agricultores que participan en el programa de mejora, llevándose a cabo actualmente un gran número de ensayos de selección-adaptación a condiciones específicas de cultivo por parte de más de 20 agricultores colaboradores.

## CONCLUSIONES

El programa de mejora de variedades tradicionales iniciado en 1998 está en su última etapa. Se dispone de líneas de los tipos De la pera y Muchamiel resistentes a ToMV, TSWV y TYLCV que han recuperado gran parte de las características morfológicas y organolépticas de las variedades originales. Esos materiales está siendo cultivado por agricultores de distintas zonas del sureste de España, en particular de la Vega Baja del Segura con éxito, pues están vendiendo sus producciones a muy buen precio. Estas colaboraciones posibilitarán el desarrollo final de distintas variedades resistentes adaptadas a muy diferentes condiciones de cultivo.

## AGRADECIMIENTOS

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# Anecoop

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Durante la Jornada de puertas abiertas, celebrada el pasado 25 de abril, los responsables de Anecoop y La Viña presentaron el Plan Estratégico de la bodega para los próximos años, centrado en tres aspectos fundamentales: potenciar la imagen de empresa, incrementar su rentabilidad y crecer un 20% en las ventas de vino de gama media-alta.
En la campaña 2006/2007 la bodega ha concentrado una producción media de 11´5 millones de kilos de uva y ha embotellado cerca de 5 millones de litros de vino bajo la D.O. Vinos de Valencia (4´5 millones de botellas y 500.000 en el nuevo formato Bag-in-Box, un envase que conserva al vacío las cualidades del vino). La facturación obtenida ha alcanzado los 7 millones de euros.
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## VALORACION DE DAÑOS OCASIONADOS POR LOS TEMPORALES DE PEDRISCO Y LLUVIA DEL MES DE MAYO

Los temporales de lluvia y pedrisco que se ha registrado durante el mes de mayo han afectado a producciones aseguradas de distintas Comunidades Autónomas con un balance, hasta la fecha de 19.572 siniestros declarados, que afectan a más de 109.734 parcelas, lo que supone una superficie siniestrada de 195.765 hectáreas.

Entre las Comunidades más castigadas se encuentran Aragón, Catalunya, Navarra, La Rioja y Castilla La Mancha, con el 60% de los siniestros declarados y con más del 75% de la superficie siniestrada. Los cultivos más dañados son uva de vino, cultivos herbáceos y frutales.

A nivel provincial destacan los daños causados en la cereza en las provincias de Cáceres y Alicante con más de 1.250 siniestros declarados y una superficie siniestrada de 7.343 hectáreas.

Las valoraciones que se abordan desde el primer momento, están siendo efectuadas por profesionales libres, expertos en los cultivos afectados, conforme a lo establecido en la Norma General y Específica de peritación para cada cultivo.

Dada la magnitud de los siniestros, Agroseguro ha establecido un plan de trabajo donde más de 230 peritos intervienen en los trabajos de valoración, lo que supone estar presentes actualmente en todas las zonas con daños.

Agroseguro recomienda a los agricultores asegurados que envíen el parte de siniestro lo antes posible, para agilizar la tramitación de las valoraciones y dar una respuesta rápida y eficaz a los asegurados afectados.

Más información:
**AGROSEGURO.**
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# Quantitative analysis of flavour volatiles detects differences among closely related traditional cultivars of tomato

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**Abstract: Volatile compounds with a major contribution to aroma have been quantitatively determined in four traditional tomato cultivars and one commercial F1 hybrid. One of the traditional cultivars was the most appreciated for flavour and overall acceptability in tests performed using a panel of 30 untrained tasters. The same cultivar showed significantly higher contents of hexanal and *cis*-3-hexenal volatile compounds, which have been previously reported to be two of the most important contributors to tomato flavour. On the basis of a small number of fruits per cultivar, significant differences among very closely related tomato cultivars can be detected for volatile aromas, thus allowing the use of the determination of volatiles as a possible tool in tomato breeding programs, making even the selection of single plants possible.**


**Keywords:** volatile aroma constituents; tomato; quantitative analysis; traditional cultivars

## INTRODUCTION

Poor flavour in tomato fruit is a serious consumer concern. It could be said that tomato flavour has declined as variety selection and tomato production has emphasized yield, fruit size, firmness, lack of defects, disease resistance and processing performance (°Brix, consistency), and not the sensory aspects of fruit quality.[1,2] With the availability of tomato all the year round and with the spread of long shelf-life varieties, consumers have begun to complain about tomato flavour. Indeed, consumers frequently associate recent varieties with a lack of flavour, although such an association has not been proved.[3] Sugars and organic acids are major components of tomato fruit and account for *ca* 60% of dry matter. They contribute to soluble solids (°Brix) and are essential factors in overall flavour intensity.[4,5] Flavour is also a function of aroma components, and it is clear that the aroma of tomatoes plays an important role in consumer acceptability.[6] However, the importance of taste and aroma in tomato flavour has never been firmly established.[7] Volatile compounds contribute to the tomato overall aroma intensity and numerous studies have been devoted to identifying the major constituents responsible for tomato aroma. Some fruits or vegetables have one or two odour-impact compounds that dominate the flavour of that particular commodity. This is not the case for tomato, however, since no single compound has been found in this fruit that is reminiscent of a ripe tomato.[8] Over 400 compounds have been identified in tomato,[9] and some of them, such as *cis*-3-hexenal, *trans*-2-hexenal, 1-hexanal, *cis*-3-hexan-1-ol, hexanol, 2-isobutylthiazole and 6-methyl-5-hepten-2-one, are considered important flavour-contributing agents.[10,11] However, the effect of genetic variation and growing conditions of tomato on aroma compounds is not well understood. Reasons for this lack of information are the complexity of analysis of volatiles,[10] the difficulty in developing a consistent methodology for sensory evaluation, and the challenge to link these analytical tools to well-defined raw materials.[12] In addition, little quantitative data is available on tomato flavour volatiles.

The tomato was probably domesticated in Mexico, although the first transfer of varieties to Europe

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was made by Spanish explorers.[13] Spain and Italy were the first European countries in which the tomato gained agricultural importance. Starting with its introduction into Spain, tomato began a process of diversification and adaptation to the different agroclimatic conditions of different localities. In this process of selection–differentiation, special attention was paid to organoleptic quality, and a great array of traditional tomato cultivars have originated in many Spanish regions.[14] Several traditional cultivars still survive in the orchards of southern Spain. They are frequently ignored outside their production area, but all of them are highly esteemed by local people due to their excellent quality. In local markets, traditional cultivars sell for three to six times the price of the hybrid varieties, as is the case for two types of local cultivars, the 'Muchamiel' and the 'De la pera'. Although cultivated tomato has a very narrow genetic base,[15] there is a considerable diversity of cultivars which differ in characteristics such as shape, firmness, soluble solid contents, etc. For example, we have found considerable levels of diversity for micronutrient content between different forms of the 'Muchamiel' and the 'De la pera' cultivars.[16] It would be interesting to find out whether there is also variability of the volatile aroma compounds. In the present study we have used a system specifically designed for the analysis of volatile organic compounds in water, called the purge-and-trap sample concentrator, which has been set up to quantify the volatile aroma constituents of fruits.[17] Among the high number of volatiles identified in tomato, a few make a major contribution to aroma, such as hexanal, *cis*-3-hexenal *trans*-2-hexenal, *cis*-3-hexenol and hexanol.[6,10,18,19] These are the compounds that show highest differences in level between varieties,[3,7] and the compounds most influenced by factors such as storage[20] or water supply.[21] The objective of this study is to try to find the differences among closely related traditional tomato varieties for some important volatile compounds. The development of a simple procedure to determine volatile aroma concentrations could be very useful as a possible tool in tomato breeding programmes for selecting genotypes with better quality characteristics.

## EXPERIMENTAL

### Plant material and growing conditions

Four traditional varieties, two of the 'Muchamiel' type (MUCH4 and MUCH18), two of the 'De la pera' type (PER1 and PER5), and the most frequent commercial variety grown in the area (BOND, an F1 hybrid cultivar distributed by the commercial company Seminis Iberica SA), were grown in hydroponics in greenhouses under homogeneous conditions, over an autumn–winter growing cycle. Fruits of the 'Muchamiel' cultivars are large in size (>200 g), flattened and strongly ribbed, while those of the 'De la pera' type weigh between 100 and 200 g, varying from rectangular to an elongated-oval shape, without ribs.

### Samples

Fruit in the same stage of ripening, with >90% of the surface showing red colour, were harvested for the five cultivars. Fruits were randomly separated into two groups for chemical and sensory analysis.

### Organoleptic tests

For sensory analysis fruits were washed and cut into wedges. The organoleptic quality of four cultivars (MUCH4, MUCH18, PER5 and BOND) was evaluated through the panel difference method. The tests were conducted according to the ranking method.[22] An incomplete-block design was used,[23] with 40 blocks of three samples per block. Using an index of 1 (bad) to 3 (good), 30 untrained tasters ranked the three fruit samples from the four cultivars, according to flavour, texture and overall acceptability. Significant differences between cultivars were determined by comparing the Durbin statistic to the $X^2$ value at $t$-1 degrees of freedom, with $t = 4$.

### Analytical determinations

Based on previous experiments, four fruits per variety were individually analysed. Three repeated analytical measurements were made for each fruit. Immediately after the fruits had been juiced, using a domestic juice extractor, 10 ml samples were frozen and stored at −81 °C until they were analyzed for volatile composition. The soluble solids content (SSC) was determined in the remaining juice with an Atago PR-100 digital refractometer (Atago Co Ltd, Tokyo, Japan), the results being expressed in °Brix. Total acidity (TA) was measured by titration with 0.1 M NaOH, and presented as g $kg^{-1}$ of citric acid. Fruit juice colour was measured with a Minolta CR-200 colorimeter (Minolta Camera Co Ltd, Osaka, Japan) adapted for juice, in order to obtain the CIELAB $L$, $a$ and $b$ parameters. Parameter $a$ is a green-to-red scale, and $b$ a blue-to-yellow scale.

### Volatile composition

A system specifically designed for the analysis of volatile organic compounds in water, called the purge-and-trap sample concentrator, has been set up to quantify the volatile aroma constituents of tomato fruits. The purge-and-trap system shows greater sensitivity than other techniques in the analysis of the volatile aroma components, so it needs lower amounts of sample per run (less than one fruit). This allows us to study the reproducibility of the analysis as well as the variability from one fruit to another, and even to analyze different parts taken from the same fruit.[17]

*Equipment*

An OI Analytical (College Station, Texas) 4560 purge-and-trap and a Hewlett-Packard 5890 (Wilmington, Delaware) gas chromatograph were used in the analysis of volatile aroma constituents. Some of

the instrumental parameters for the purge-and-trap and gas chromatography (GC) are as follows: purge-and-trap sample, 1 ml; trap, OI Analytical #10 (Tenax/silica gel/carbon molecular sieve); purge, He at 10 psi for 11 min at 25 °C; desorption, 4 min at 180 °C; transfer line, 180 °C; valve oven, 100 °C; gas chromatography column, DB-624 (J&W Scientific, Folsom, CA) 0.25 mm × 30 m × 1.4 mm; oven, 60 °C (5 min) then 3 °C min$^{-1}$ to 220 °C (10 min); carrier gas, He at 1 ml min$^{-1}$; transfer line, 225 °C; FID, 250 °C. A flame ionization detector (FID) (Wilmington, Delaware, USA) is used to measure the separated compounds.

*Procedure*

The sample is placed in a purge-and-trap sample concentrator where an inert gas is purged through the sample. The volatiles travel out with the gas flow and are trapped onto a sorbent trap. After sample purging is completed, the trap is heated, and the volatiles are desorbed to the injector whose temperature is −100 °C (cooled with liquid nitrogen). When the desorption is finished, the injector is quickly heated to 210 °C, and the volatiles are injected 'on-column' into a gas chromatograph containing a capillary column. The separated compounds are detected by using an FID. During the GC separation the trap is baked at 180 °C with an inert gas (He) flowing in opposite direction to the purge flow. This prepares the trap for the next sample. Two repeated measurements were made for each fruit.

It is important to point out the differences between the head-space technique and the purge-and-trap system, also called 'dynamic head-space technique'. The static head-space method extracts a small amount of volatiles from a sample, since this technique only analyses the volatiles contained in the vapour phase which is in equilibrium with the liquid sample. Therefore, each peak of the chromatogram only represents a part of the total content of the sample. In contrast, the purge-and-trap technique is based on an efficient transference of all the volatile organic compounds from the aqueous to the gaseous phase by bubbling at room temperature an inert gas through a liquid sample contained in a specifically designed purging chamber. In preliminary studies, standards of each volatile compound were added to tomato samples in order to select convenient bubbling times for achieving complete transference of all the volatiles. The chromatogram peak then, represents the total content of the sample. This was true even with the compound *cis*-3-hexenal; although it is very unstable, the short time of bubbling (11 min at 25 °C) allowed total recovery of the compound. In order to check that a total transference has been achieved, no volatiles should be detected by a second bubbling using an already processed sample. This aspect of the purge-and-trap system, the total recovery of the compounds, allows the use of external standards to obtain calibration curves.[17]

Concentrations of the volatile compounds hexanal, *cis*-3-hexenal, *trans*-2-hexenal, *cis*-3-hexenol and hexanol were calculated using regression equations, determined by placing four different concentrations of each standard in the purge-and-trap sample concentrator to obtain calibration curves as described previously.[17] The logarithm of odour unit values (log odour) was calculated from the ratio of the concentration of a component to its odour threshold, using the values of threshold determined by Buttery *et al*.[24] Volatile compounds with positive odour units are assumed to contribute to the flavour of a food, while those with negative units are not.[8]

A variance components analysis (ANOVA with a nested or hierarchical design) was undertaken to estimate the amount of variability provided by each of the three factors in the experiment: variability among cultivars, among fruits within each cultivar and among repeated measurements of the same fruit. Duncan's multiple-range test was used to establish possible significant differences among the volatile content of the different cultivars.

## RESULTS AND DISCUSSION

### Organoleptic tests

Tomato fruits from the 'Muchamiel' type are clearly distinguishable from those of the 'De la pera' type and from those of the hybrid variety on the basis of morphological characteristics, but fruits from cultivars of the same type are very similar and it is impossible to visually differentiate between them. However, in previous experiments all the cultivars analyzed in the present work have differed in yield characteristics (data not shown) and in some analytical parameters, such as micronutrient content.[16] The results of the organoleptic test presented in Table 1 suggest that these cultivars also differ in sensory aspects of fruit quality. Although the test panel was made up of untrained tasters, significant differences for flavour and overall acceptability, but not for texture, were detected. The cultivar PER1 was not included in the test in order to reduce the number

**Table 1.** Flavour, texture, and overall acceptability values for the four cultivars evaluated

| | Flavour | | Texture | | Overall acceptability | |
|---|---|---|---|---|---|---|
| Cultivar[a] | Score[b] | Mean | Score | Mean | Score | Mean |
| MUCH4 | 71 | 2.4 | 64 | 2.1 | 74 | 2.5 |
| MUCH18 | 58 | 1.9 | 66 | 2.2 | 57 | 1.9 |
| PER5 | 66 | 2.2 | 60 | 2.0 | 63 | 2.1 |
| BOND | 47 | 1.6 | 55 | 1.8 | 53 | 1.8 |
| T value[c] | 12.375** | | 2.89$^{NS}$ | | 9.86** | |

[a] Only one of the two cultivars of the De la pera type was evaluated.
[b] Each value is the sum of scores of 30 tasters, on a 1 (bad) to 3 (good) scale. Maximum = 90.
[c] $T$ = Durbin statistic. ** Significant at $p \leq 0.01$. NS = not significant at $p \leq 0.01$.

of samples presented to the panellists, since a large number of samples makes the detection of differences more difficult In addition, previous tests had shown only slight sensory differences between 'De la pera' cultivars. The cultivar MUCH4 received the highest scores for flavour and overall acceptability, being significantly better even than the other cultivar of the same type, MUCH18. The second most appreciated cultivar was PER5, both for flavour and for overall acceptability. The hybrid BOND, in spite of being more adapted to greenhouse-growing conditions than the traditional varieties, received the lowest scores for the three aspects evaluated, although the differences for texture were not significant.

## Analytical determinations

*Colour parameters, SSC and TA*

We are trying to develop a simple analytical procedure to compare quantitatively desirable flavour volatiles in tomato lines, since it would be a useful tool in selecting for a better tasting tomato. For practical reasons, fruits were visually selected on the basis of external colour, trying to obtain the most uniform stage of maturity for the fruits of each of the five genotypes. A variance components analysis was then performed on simple maturity analytical parameters to estimate the amount of variability provided by the factors 'cultivars', 'fruits within each cultivar' and 'error' (repeated measurements of the same fruit) (Table 2). For the juice colour parameters, as expected, cultivar was the factor that gave rise to most variance. There was low variability among fruit samples and very low variance due to the error. In spite of the fruits being selected according to a homogeneous degree of maturity, for SSC and TA the amount of variability among fruits was important and higher than the variation associated with differences among cultivars. This result should be taken into account when considering volatile aroma analysis, since volatile aroma content also varies with maturity.[8,25] Part of the variability for the volatile compounds concentrations will be due to inevitable differences among the internal maturity stages of the fruits analyzed.

*Volatile compounds*

Figure 1 shows a typical purge-and-trap/GC pattern of tomato fruit. Differences among varieties represented between 23 and 52% of the total variation for volatile contents. Between 5 and 47% of the variability was due to differences among fruits of the same cultivar, and the error associated to the analytical determination ranged from 21 to 72% (Table 2). This analytical error was particularly high for the compound *trans*-2-hexenal. Due to the complexity of volatile compounds analysis, there is always a high intrinsic variability associated with quantitative data, as in this case. However, the ANOVA detected significant differences among cultivars for all the analyzed compounds ($p < 0.05$). The differences for *trans*-2-hexenal, *cis*-3-hexenol and hexanol were very low, and those for hexanal and *cis*-3-hexenal were of greater importance.

Data available in the scientific literature about quantitative analysis of tomato volatiles, in addition to being scarce, shows values over a wide range. This may be partially due to differences in the plant material analysed, but the main factor is probably the analytical method used by the different authors. For example, the values we have obtained for hexanal content (ranging from 2.33 to 5.16 mg kg$^{-1}$) are similar to the 3.1 mg kg$^{-1}$ cited by Buttery *et al*[26] in fresh tomato, although others authors give values ranging from 0.05 to 12.3 mg kg$^{-1}$.[27,28] For *cis*-3-hexenal content, our data varies between 1.51 and 2.47 mg kg$^{-1}$, while data reported by other authors ranged from 0.004 to 12 mg kg$^{-1}$.[26,29] The same is true for the other compounds analyzed.

All the cultivars were grown under greenhouse conditions over an autumn–winter cycle and, as mentioned before, the modern variety BOND should be more suited to these growing conditions. However, BOND showed the lowest concentrations of all the compounds analyzed (although BOND yield under greenhouse is two to three times that of traditional cultivars). For some compounds we have found significant differences even between cultivars of the same type. For example, the cultivar MUCH4 showed higher content for the hexanal and *cis*-3-hexenal

**Table 2.** Variance components (%) obtained from a nested ANOVA for the analytical parameters

| | | Colour parameters, SSC and TA | | | | |
|---|---|---|---|---|---|---|
| Source of variation | *df* | a | b | L | SSC | TA |
| Among cultivars | 4 | 73.4 | 48.8 | 81.6 | 40.6 | 29.2 |
| Among fruits | 15 | 25.8 | 44.2 | 15.0 | 55.6 | 66.5 |
| Error | 40 | 0.8 | 7.0 | 3.4 | 3.8 | 4.3 |
| Total | | 100 | 100 | 100 | 100 | 100 |
| | | Aroma volatiles | | | | |
| Source of variation | *df* | Hexanal | *cis*-3-Hexenal | *trans*-2-Hexenal | *cis*-3-Hexenol | Hexanol |
| Among cultivars | 4 | 52.0 | 25.3 | 23.3 | 28.0 | 30.6 |
| Among fruits | 15 | 26.7 | 24.1 | 5.0 | 24.4 | 46.8 |
| Error | 20 | 21.3 | 50.6 | 71.7 | 47.6 | 22.6 |
| Total | | 100 | 100 | 100 | 100 | 100 |

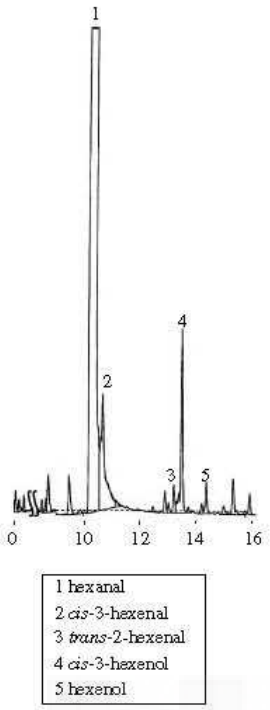


**Figure 1.** Purge-and-trap/GC pattern of tomato fruit, cultivar MUCH4.

compounds than the two 'De la pera' cultivars and MUCH18. Cultivars PER1 and PER5 showed the highest content of *trans*-2-hexenal, *cis*-3-hexenol and hexanol, although the differences were not important and in only a few cases were statistically significant (Table 3). All the compounds analyzed showed positive odour units (were present at a concentration above their threshold) and, according to Baldwin *et al*,[8] are assumed to contribute to the flavour of the fruits. None the less, because of possible interactions with other compounds, odour unit values might not give a clear indication of an individual aroma compound's contribution when in a complex mixture.[2]

Using principal component analysis,[21] relationships between aroma volatiles and sensory attributes determined by quantitative description analysis were found in tomatoes. *cis*-3-Hexenal, the most odour-active compound, was associated with the flavour attributes 'fruity' and 'sweet'. This compound is probably the most important contributor to tomato aroma and flavour.[6,30] The hexanal odour has been described as 'green, herbaceous'. In three seasonal studies, sweetness intensity was related to hexanal, with contributions from *cis*-3-hexenal, *trans*-2-hexenal or *cis*-3-hexenol.[8]

The fact that MUCH4, the most appreciated cultivar in the organoleptic test, was also the cultivar with the higher content of hexanal and *cis*-3-hexenal, two of the most important contributors to tomato aroma, is a suggestive result. However, we should not forget that both the absolute concentrations of sugars and organic acids and the balanced ratio between them are also important factors in consumer acceptance. Increasing total sugar and organic acid levels of fresh tomato improved flavour acceptability,[31,32] and a balanced sugar/organic acid ratio was preferred by a panel examining the flavour characteristics of cherry tomato.[33] However, since we have found no important differences between cultivars for SSC and TA, it is therefore more likely that the contribution from the aroma of volatiles was more important in the panellist appreciation. In this sense, Baldwin *et al*[7] found that tomato-like flavour intensity ratings for seven cultivars were almost identical to the rating for overall acceptability, indicating the close relationship between flavour and tomato quality.

The situation, however, is not so straightforward; even after extensive research on tomato flavour compounds there is little definitive information on the relationship between flavour/aroma compounds and sensory flavour perception.[2] Baldwin *et al*[7] conducted extensive research to find correlations between sensory data and measurements of volatile compounds, but their conclusions were not definitive. Thus far, flavour quality for tomato has been an elusive trait and we still lack quantifiable definition for tomato flavour.[8] There is no complete agreement about which flavour compounds are important and what the appropriate levels and balance for good flavour are. For example, the recommendations of Tandon *et al*[30] directly contradict those of Gray *et al*,[34] who recommended increasing levels of C6 aldehydes. Its is likely that the desirable levels of

**Table 3.** Volatile compounds concentration (mg kg$^{-1}$) and their log odour units (log *U*)

| | Hexanal | | *cis*-3-Hexenal | | *trans*-2-Hexenal | | *cis*-3-Hexenol | | Hexanol | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | mg kg$^{-1}$ | log *U* | mg kg$^{-1}$ | log *U* | mg kg$^{-1}$ | log *U* | mg kg$^{-1}$ | log *U* | mg kg$^{-1}$ | log *U* |
| MUCH4 | 5.16$^{a}$ | 3.06 | 2.47$^{a}$ | 3.99 | 0.93$^{b}$ | 1.74 | 0.96$^{b}$ | 1.14 | 0.73$^{b}$ | 0.16 |
| MUCH18 | 3.35$^{b}$ | 2.87 | 1.93$^{ab}$ | 3.89 | 0.96$^{b}$ | 1.75 | 0.98$^{b}$ | 1.15 | 0.77$^{a}$ | 0.19 |
| PER1 | 3.26$^{b}$ | 2.86 | 1.01$^{c}$ | 3.61 | 1.03$^{ab}$ | 1.78 | 1.03$^{ab}$ | 1.17 | 0.79$^{a}$ | 0.20 |
| PER5 | 3.61$^{b}$ | 2.90 | 0.89$^{c}$ | 3.55 | 1.13$^{a}$ | 1.82 | 1.12$^{a}$ | 1.20 | 0.78$^{a}$ | 0.19 |
| BOND | 2.33$^{c}$ | 2.71 | 1.51$^{bc}$ | 3.78 | 0.91$^{b}$ | 1.73 | 0.93$^{b}$ | 1.12 | 0.71$^{b}$ | 0.15 |
| SD | 0.58 | | 1.04 | | 0.12 | | 0.11 | | 0.05 | |

Values followed by different letters within a column are significantly different at $p < 0.05$ (Duncan's test). SD: standard deviation; sample size: $n = 40$.

the different volatiles depend on the target market. In the southeast of Spain consumer preferences for traditional cultivars varies even between neighbouring regions. For future breeding purposes, consumer testing might be required to determine which are the levels of compounds that would enhance the acceptability of tomatoes.

Poor flavour quality in tomato appears to be, in part, a result of breeding practices that do not select for flavour, because of lack of information.[8] Sensory parameters that could assist the breeders in an efficient selection for flavour have not been characterized. The definition and use of markers that correlate with tomato flavour could improve this situation and provide the breeder and processor with analytical tools for flavour enhancement.[12] Breeders could also use sensory analysis, but this is often difficult to perform and requires access to a panel and considerable expertise.[8] Quantitative comparison of desirable flavour volatiles, in addition to reducing sugars and free acids, in tomato breeding lines would be a useful tool in selecting for a better tasting tomato. However, several authors have reported that differences among tomato cultivars for volatile compounds are not important. For example, Buttery *et al*[11] found no marked differences for the concentrations of volatiles of more than 10 different tomato commercial lines. The main quantitative differences seemed to be caused by variations in degrees of ripeness or by the storage conditions. In contrast, Baldwin *et al*[8] have reported significant differences between cultivars in levels of important aroma compounds. The different analytical methods used could probably justify these discrepancies. Our results show that the analytical method we used is valid to detect quantitative differences among tomato varieties. It is particularly interesting that we were able to detect differences among very closely related cultivars of the same type. Modern genetic and genomic tools are currently being intensively applied to the tomato. Simple sequence repeat (SSR) or microsatellites are becoming the preferred molecular markers in crop breeding, and they are the most practical markers for variety identification. He *et al*[35] have recently found that the combination of only five selected SSR loci could differentiate all the 19 tomato cultivars they were studying. However, using these five SSRs we have found no differences between the cultivars MUCH4, MUCH 18, PER1 and PER5 (Ruiz JJ unpublished), indicating the great similarity among them.

## CONCLUSIONS

Methods to analyze volatile compounds that need large amounts of tomato samples are not useful for selecting individual genotypes. As analysis of flavour compounds in the aromatic component requires expensive equipment and training; if determination of volatiles is to be used as a tool in selection programs, a low number of samples should be needed. We have found significant differences among closely related cultivars for selected volatile compounds using a small number of fruits per cultivar whose maturity stage had been visually judged. Tomato aroma is complex, probably a combination of more than 16 compounds give tomato its unique odour characteristics. However, reducing the number of compounds to a few with major contributions to aroma could increase the usefulness of volatile determinations in tomato breeding programmes. In this sense, although further investigations are needed, it is interesting that the most appreciated cultivar in the organoleptic tests was the one with high content of the volatile compounds hexanal and *cis*-3-hexenal.

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# Characterization of Spanish Tomatoes using Aroma Composition and Discriminant Analysis

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Nowadays, tomato breeders are trying to associate high fruit firmness, long shelf life, high disease resistance, and *good flavor*; however, breeding for sensory quality has been severely restricted up to date. In this study, a system specifically designed for the analysis of low amounts of material has been set up and tested to quantify the volatile aroma constituents of tomatoes. Ten volatile compounds with a major contribution to tomato aroma have been quantitatively determined in two traditional tomato types (*Muchamiel* and *De la Pera*) and one hybrid type (*Odissea*). Both traditional types presented higher intensities of tomato odor and aroma according to a trained panel and they were more accepted by a consumer panel than the hybrid tomatoes. The traditional tomatoes showed significantly higher contents of most of the volatiles studied. Significant differences among traditional and hybrid types were found and a mathematical model that successfully discriminated among tomato types was developed using only the concentrations of six volatile compounds: 3-methylbutanal, 1-penten-3-one, hexanal, *trans*-2-hexenal, 1-hexanol, and 2-isobutylthiazole. This mathematical model could help in using volatile determination as a possible tool in tomato breeding programs and in maintaining and improving traditional Spanish tomato cultivars.

*Key Words:* tomatoes, *Lycopersicon esculentum*, odor, aroma, traditional cultivars

## INTRODUCTION

The considerable efforts of tomato breeders have mainly emphasized yield, fruit size, fruit appearance (lack of defects and attractive color), disease resistance and, more recently, fruit firmness and shelf life to allow long-distance trading (Saliba-Colombani et al., 2001). However, lack of characteristic tomato flavor is a common complaint among consumers when purchasing tomatoes especially in areas that are far from tomato growing regions (Baldwin et al., 1998). According to a market survey, the sensory quality of a food product has been ranked higher than its nutritional value, price or safety, from a consumer point of view (Saliba-Colombani et al., 2001).

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Tomato was probably domesticated in Mexico and brought to Europe by Spanish explorers (Rick et al., 1974). At this time, tomato began a process of diversification and adaptation to the different agro-climatic conditions of different Spanish regions. In this process of selection differentiation, special attention was paid to sensory quality, and a great array of traditional tomato cultivars were originated (Gómez et al., 2001). Several traditional cultivars still survive in the orchards of Southern and Eastern Spain. They are frequently ignored outside their production area, but all of them are highly esteemed by local people due to their excellent sensory quality. In fact, in local markets, traditional cultivars are sold for three to six times the price of the hybrid varieties, as is the case for two types of local cultivars (Valencian Community, Eastern Spain), the 'Muchamiel' and the 'De la Pera' types. Although cultivated tomato has a very narrow genetic base (Picó et al., 2002), there is a considerable diversity of cultivars, which differ in characteristics such as shape, firmness, soluble solid contents, etc. For example, it was found considerable levels of diversity for micronutrient content among different forms of the 'Muchamiel' and the 'De la Pera' cultivars (Ruiz et al., 2005c).

Organoleptic quality involves taste and aroma (flavor), color and texture of fruit. Many studies on

tomato described the chemical composition of the fruit (Davies and Hobson, 1981). Flavor mainly depends on sugar and acid contents but also on the sugar/acid ratio. However, the weak correlation between the tomato-like flavor and sugar and acid contents justified the study of volatile composition. Many researchers have identified and quantified aroma volatiles essential to tomato flavor (Petro-Turza, 1986). Analysis of tomato aroma was greatly facilitated by the combined used of gas chromatography (GC) and mass spectrometry (MS), particularly the isolation and identification of volatiles. More than 400 aroma volatiles have been identified in tomato fruit (Petro-Turza, 1986). Buttery (1993) reported on volatiles present at concentrations greater than one part per billion in tomato fruit (narrowing >400 volatile down to 30), and did odor threshold studies to determine which of these are likely to contribute to tomato aroma. In fact, Buttery (1993) prepared a synthetic mixture consisting of just 10 volatiles (*cis*-3-hexenal, *cis*-3-hexanol, hexanal, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and $\beta$-ionone) and sensory panel studies showed this mixture to have an aroma very similar to that of sliced fresh tomato (Buttery et al., 1990).

The influence of variety (Langlois et al., 1996), ripening stage (Baldwin et al., 1991) and storage conditions (Stern et al., 1994) on the most important aroma volatiles was proved, but little is known about their genetic control. Breeding for sensory quality has been severely restricted by the lack of efficient criteria and by the polygenic nature of the trait. One of the present preoccupations of tomato breeders is to associate high fruit firmness, shelf life, a good flavor, and many disease resistance genes in the same variety. For this purpose, both efficient and easy to measure criteria are needed. Sensory analysis can efficiently describe flavor and texture, but is not of practical use for breeders since large trained panels are necessary for several weeks of analysis. Physical and chemical methods can also be used to evaluate components of texture and flavor, like fruit firmness, and the contents of sugars, acids, and aroma volatiles (Saliba-Colombani et al., 2001).

In a previous study, our team demonstrated that the determination of volatiles, using a purge-and-trap system, can be used to differentiate among closely related traditional cultivars of Spanish tomatoes (Ruiz et al., 2005a). In this way, it was showed that determination of the main volatiles of tomatoes could be used as a possible tool in tomato breeding programs, making possible even the selection of single plants due to the small amount of sample needed for the assays in this chromatographic technique.

The global aim of this study was to demonstrate that genetic improvement should consider sensory parameters and that instrumental measurement of volatile compounds could help in this way. With this general idea in mind, the specific objective was to develop a mathematical model (discriminat analysis) that could help breeders to classify fruits according to its tomato type (group of tomato cultivars with similarities in at least one parameter, for instance shape of the fruits) using the concentrations of 10 volatiles (quantified by the purge-and-trap system). This mathematical model will be of help especially in identifying hybrid fruits, which could be similar in shape to the traditional types (*Muchamiel* and *De la Pera*).

## MATERIALS AND METHODS

### Material

*Plant Material and Growing Conditions*

Plants of two traditional cultivars of the *Muchamiel* type (MUCH9 and MUCH18), two cultivars of the *De la Pera* type (PER7 and PER10), and one of the most frequent commercial variety grown in the area (*Odissea*, a F1 hybrid cultivar distributed by the commercial company Seminis Iberica S.A.) were grown in a hydroponic greenhouse under homogeneous conditions, over an autumn winter growing cycle in Orihuela, Alicante (Eastern Spain). Tomatoes were harvested on November 25, 2006. Fruits of *Muchamiel* and *Odissea* cultivars are large in size (>200 g), flattened and strongly ribbed, while those of the *De la Pera* type weight between 100 and 200 g, varying from rectangular to an elongated-oval shape, without ribs.

*Samples*

Tomatoes were picked when the parameters such as color, texture, acidity, and soluble solids content were those required for the optimum commercial harvest in the Valencia area (Eastern Spain). For instance, fruit in the same stage of ripening, with >90% of the surface showing red color, were harvested for the three cultivars. Samples for analyses were obtained from ten homogeneous samples of 1 kg of tomatoes (5 6 pieces), picked from the sunny side of the plants and at the same height; therefore, 10 kg of product were initially available for each tomato type. Tomatoes were picked at random from different plants of each plot. Fruits were randomly separated into two groups, for chemical and sensory analysis.

*Quality Parameters*

Based on previous experiments, 10 fruits per tomato type were individually analyzed (three repetitions per fruit). Immediately after the fruits had been juiced, using a domestic juice extractor, the total soluble solids concentration (TSS) was determined with an Atago

PR-100 digital refractometer (Atago Co Ltd, Tokyo, Japan), the results were expressed as °Brix. Titratable acidity (TA) was measured by titration with 0.1 N NaOH using phenolphthalein until pH 8.1, and presented as percentage of citric acid. Fruit juice color was measured with a Minolta CR-300 colorimeter (Minolta Camera Co Ltd, Osaka, Japan) adapted for juice, in order to obtain the CIEL*a*b* parameters.

*Extraction Procedure of Volatile Aroma Compounds*

A system specifically designed for the analysis of volatile organic compounds in water, called the purge-and-trap sample concentrator, has been set up to quantify the volatile aroma constituents of tomato fruits. The purge-and-trap system shows greater sensitivity than other techniques in the analysis of the volatile aroma components, so it needs lower amounts of sample per run (less than one fruit). This allows researchers to study the reproducibility of the analysis as well as the variability from one fruit to another, and even to analyze different parts taken from the same fruit (Ruiz-Beviá et al., 2002). Analysis of headspace flavor volatiles was done by GC-MS.

*Chromatographic Analyses*

A Tekmar-Dohrman (Emerson, Mason, OH) 3100 purge-and-trap and an Agilent 6890 N Network (Agilent Technologies, Inc., Santa Clara, CA) gas chromatograph were used in the analysis of volatile aroma constituents. Some of the instrumental parameters for the purge-and-trap and GC are as follows: purge-and-trap sample, 2 mL; trap, tenax/silica gel/carbon molecular sieve; purge, He at 10 psi for 11 min at 70 °C; desorption, 4 min at 220 °C; transfer line, 225 °C; valve oven, 150 °C; GC column, DB-624 (J&W Scientific, Folsom, CA) 0.25 mm × 30 m × 1.4 μm; oven, 60 °C (5 min) then 3 °C/min to 220 °C (10 min); carrier gas, He at 0.9 mL/min; transfer line, 225 °C. An Agilent 5973 Network MS detector was used to identify the isolated aroma compounds. Mass spectra were obtained by electron ionization (EI) at 70 eV, and spectra range of 30 350 m/z was used.

Concentrations of 10 volatile compounds (3-methylbutanal, 1-penten-3-one, hexanal, *cis*-3-hexenal, *trans*-2-hexenal, *cis*-3-hexenol, 1-hexanol, 6-methyl-5-hepten-2-one, 2-isobutylthiazole, and methyl salicylate) were calculated using regression equations, determined by injecting four different concentrations of each standard to obtain calibration curves as described previously (Ruiz-Beviá et al., 2002). Three repeated measurements were made for each fruit. The logarithm of odor unit values (log odor) was calculated from the ratio of the concentration of a component to its odor threshold, using the values of threshold determined by Buttery et al. (1971). Volatile compounds with positive odor units are assumed to contribute to the flavor of a food, while those with negative units are not (Baldwin et al., 2000).

*Cis*-3-Hexenol was obtained from Panreac (Barcelona, Spain), all the rest of standards were purchased from Sigma-Aldrich (Milwaukee, WI, USA).

*Sensory Evaluation with Trained Panel*

Sensory evaluation with trained panel was used to discriminate the intensities of 'fresh tomato' odor (perception of volatile compounds sniffed through the nose) and aroma (perception of volatile compounds during chewing of samples).

A panel of 10 panelists, ages 20 50 years (6 female and 4 male, all members of the University Miguel Hernandez) with sensory evaluation experience, was trained in descriptive evaluation of tomato.

The panel was selected and trained following the ISO standard 8586-1 (AENOR, 1997). For the selection of the panel members, and after rejecting candidates with obvious drawbacks, ranking/rating tests for intensity of acid, sweet, and tomato volatile compounds were carried out. These tests were used to determine candidates' ability to discriminate and describe graded levels of intensity of a given attribute. Series of samples in random order were presented to candidates, in which one parameter is present at different levels, which cover the range present in the products of interest. Candidates ranking samples correctly or inverting only adjacent pairs were selected; the same rank order criterion was used for the rating tests (AENOR, 1997; Meilgaard et al., 1999).

Training sessions were carried out twice a week for 1 month, that is, 10 training sessions of 2 h were needed (20 h). The amount of time needed depends on the complexity of the product, on the experience of the panelists and their knowledge on the product, on the number of attributes to be covered, and on the requirements for validity and reliability (Meilgaard et al., 1999). All panelists selected had more than 1 year's experience in discrimination and descriptive test on a variety of foods. The structure of the training period was as follows: (a) terminology development (3.5 h); (b) introduction to descriptive scaling (3 h); (c) initial practice (5.5 h); (d) small product differences (2.5 h); and, (e) final practice (5.5 h). The tomatoes used during the training sessions have been grown in the UMH's greenhouses facilities (Orihuela, Alicante) and were from several different cultivars and growing cycles.

Further details about panel selection and training could be found in Ruiz et al. (2005b).

Measurements were performed in individual booths with controlled illumination and temperature (AENOR, 1997; Meilgaard et al., 1999).

The individual products were scored for tomato sensory parameters on a scale of 0 5, where: 0 = no odor/aroma is perceived; 1 = extremely slight odor/aroma;

2 = slight odor/aroma; 3 = regular odor/aroma of fresh tomatoes; 4 = intense odor/aroma; 5 = extremely intense odor/aroma.

Panelists relied on their training experience to score products and tomatoes were presented as fresh wedges in 50 mL plastic beakers with lids. The entire experiment was repeated three times (all judges scored the three tomato type samples on each session for a total of three sessions) and the sensory scores were presented as the overall means. Therefore, each data on Table 2 was the mean of 30 evaluations (10 assessors × 3 repetitions).

*Sensory Evaluation with Consumers*

Besides, 30 untrained assessors (Stone and Sidel, 1993), regular consumer of fresh tomatoes, quantified their overall acceptability of three tomatoes of the three different tomato types (even though three tomatoes were provided to each consumer just one score was finally obtained). Therefore, each hedonic data on Table 2 was the mean of 90 evaluations (30 consumers × 3 repetitions). Consumers expressed their acceptability using a scale of 0 (none) to 10 (extremely high); this scale was selected because Spanish people are very familiar with it, for instance school marks are given using this scale.

*Statistical Analysis*

After checking that data from the rating experiments fitted a normal distribution (Cochran-Bartlett test) these data were subjected to two-way (assessor, tomato type, and interaction) ANOVA (analysis of variance) and the Tukey's least significant difference multi-comparison test to determine significant differences among samples.

Discriminant analysis was used to classify aroma profiles from different tomato types into preexisting categories. A mathematical function was developed using the set of continuous independent variables (concentrations of the 10 volatiles studied) that best discriminated among the category (tomato type) from which the items arise (Meilgaard et al., 1999).

All statistical analyses were carried out using SPSS 11.5 for Windows (SPSS Science, Chicago, USA).

## RESULTS AND DISCUSSION

Data on Table 1 show that maturity of the original fruits was quite uniform and that any effect on aroma composition should only be due to the tomato type and not to quality parameters at harvest. The mean values (all tomato types) for TSS, TA, maturity index, and the green-red coordinate (a*) were 7.43 ± 0.25 °Brix, 1.75 ± 0.08% citric acid, 4.24 ± 0.27 and 7.38 ± 1.07, respectively.

**Sensory Evaluation**

In previous experiments all tomato types analyzed in the present work have shown to differ in yield characteristics and in some analytical parameters, such as micronutrient content (Ruiz et al., 2005c). Results of the sensory tests presented in Table 2 suggested that these tomato types also differed in sensory attributes of fruit quality. The panel of trained assessors found significant differences for both 'odor' and 'aroma'. However, no significant differences were found for assessors and interaction, which meant that all of them were scoring in a coherent way and that the only effect on odor/aroma perception was due to the tomato type. The traditional types *De la Pera* and *Muchamiel* received higher scores for both odor and aroma than the hybrid type; although no significant differences were found between *De la Pera* and *Muchamiel*. In a previous study (Ruiz et al., 2005a) carried out with similar cultivars of tomato, one of the traditional cultivars, MUCH4, was the most appreciated for flavor and acceptability in tests performed using a panel of 30 untrained assessors.

**Table 1.** Fruit quality values, at harvest of *Muchamiel*, *De la Pera*, and *Odissea* tomatoes.

| | Mean of 30 values (10 fruits × 3 repetitions) | | | |
|---|---|---|---|---|
| Parameters | Muchamiel | De la Pera | Odissea | ANOVA[1] |
| TSS (°Brix) | 7.54 ± 0.18 | 7.41 ± 0.15 | 7.35 ± 0.19 | N.S. |
| TA (% citric acid) | 1.73 ± 0.06 | 1.75 ± 0.03 | 1.78 ± 0.05 | N.S. |
| Maturity Index (TSS/TA) | 4.36 ± 0.17 | 4.23 ± 0.10 | 4.13 ± 0.20 | N.S. |
| Color (a*) | 8.01 ± 0.51 | 7.16 ± 0.67 | 6.98 ± 0.58 | N.S. |

[1]N.S. = non-significant F ratio ($p < 0.05$).

**Table 2.** Odor and aroma scores by trained panel and overall acceptability by consumer panel for the three tomato types evaluated (*Muchamiel, De la Pera* and *Odissea*).

| Variation source | Odor | Aroma | Overall acceptability[3] |
|---|---|---|---|
| ANOVA test[1] | | | |
| Assessor | NS | NS | NS |
| Type | *** | *** | ** |
| Assessor × Type | NS | NS | NS |
| Tukey's Multiple Range Test[2] | | | |
| Type | | | |
| Muchamiel | 3.5 a | 3.4 a | 7.3 a |
| De la Pera | 3.6 a | 3.4 a | 7.0 a |
| Odissea | 2.5 b | 2.5 b | 5.9 b |

[1]N.S. = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. Mean of 30 evaluations (10 assessors × 3 repetitions).
[2]Values followed by the same letter, within the same source of variation, are not significant different ($p < 0.05$).
[3]Mean of 90 evaluations (30 consumers × 3 repetitions).

The consumer panel also showed a better overall acceptability of the traditional tomato types over the hybrid type (Table 2).

In summary, the hybrid *Odissea*, in spite of being more adapted to greenhouse-growing conditions than the traditional cultivars, received the lowest scores for all sensory attributes evaluated and for both trained and consumer panels.

### Volatile Aroma Compounds

Physicochemical properties of the aroma compounds in combination with the chemical nature of the food matrix ingredients and the formed matrix structure can modify the concentration of the aroma compounds in the headspace. As a result, odor activity values based on odorants' quantities in the food and the odor thresholds in water may not always be completely indicative of the actual odor potency of a particular food (Bezman et al., 2003). The compounds most affected by this situation were (*E,E*)-2,4-decadienal, β-damascenone, and β-ionone, which were not studied here. On the other hand, the big advantage of the purge-and-trap methodology is that very low amounts of material are needed to carry out the analyses. Therefore, the purge-and-trap is a completely valid and useful methodology for a comparative study of the aroma intensity of different types of tomatoes, especially if instrumental results are validated with sensory data.

Data on Tables 3 and 4 proves that 8 out of the 10 volatile compounds studied contribute to tomato flavor because they presented positive odor units (Baldwin et al., 2000). The only two compounds presenting negative odor units, and therefore not contributing to tomato flavor, were *cis*-3-hexenol and 1-hexanol.

Data available in the scientific literature about tomato quantitative volatiles analyses, in addition to being scarce, shows a great dispersion. This may be partially due to differences in the plant material analyzed (e.g., maturity and breeding), and/or the analytical method used by the different authors. For example, the values obtained here for hexanal content (ranging from 1.66 to 3.00 mg/L) agree with those previously cited by Buttery et al. (1990) in tomato paste, 3.1 mg/kg, but are lower than reported by Maul et al. (1998), 12.33 mg/kg, using a gas chromatographic headspace analysis technique. The same is true for the other analyzed compounds.

All the cultivars were grown under greenhouse conditions over an autumn winter cycle and the modern type *Odissea* should be more suited to these growing conditions and, in fact, *Odissea* yield under greenhouse is two to three times that of traditional types. However, the hybrid type showed the lowest volatile contents for almost all the analyzed compounds (Tables 3 and 4), implying a poorer organoleptic quality than the traditional tomato types studied even under greenhouse conditions.

**Table 3.** Volatile compounds concentration and their log odor units (log *U*).

| | 3-Methylbutanal | | 1-Penten-3-one | | Hexanal | | *cis*-3-Hexenal | | *trans*-2-Hexenal | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variation source | (mg/L) | log *U* | (mg/L) | log *U* | (mg/L) | log *U* | (mg/L) | log *U* | (mg/L) | log *U* |
| ANOVA test[1] | | | | | | | | | | |
| Type | *** | *** | ** | * | * | * | ** | ** | ** | ** |
| Tukey's multiple Range test[2] | | | | | | | | | | |
| Type | | | | | | | | | | |
| Muchamiel | 0.045 a | 2.34 a | 0.093 c | 1.96 b | 3.00 a | 2.78 a | 0.868 a | 3.53 a | 0.223 b | 1.16 b |
| De la Pera | 0.038 a | 2.27 a | 0.115 b | 2.06 a | 3.72 a | 2.78 a | 0.975 a | 3.58 a | 0.296 a | 1.14 b |
| Odissea | 0.027 b | 2.12 b | 0.128 a | 2.10 a | 1.66 b | 2.56 b | 0.629 b | 3.40 b | 0.310 a | 1.26 a |

[1]N.S. = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
[2]Values are the mean of three repetitions. Values followed by the same letter, within the same source of variation, are not significant different ($p < 0.05$).

**Table 4.** Volatile compounds concentration and their log odor units (log *U*).

| | *cis*-3-Hexenol | | 1-Hexanol | | 6-Methyl-5-hepten | | 2-Isobutylthiazole | | Methyl salicylate | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variation source | (mg/L) | log *U* | (mg/L) | log *U* | (mg/L) | log *U* | (mg/L) | log *U* | (mg/L) | log *U* |
| ANOVA test[1] | | | | | | | | | | |
| Type | NS | NS | ** | ** | ** | ** | NS | NS | *** | *** |
| Tukey's Multiple Range test[2] | | | | | | | | | | |
| Type | | | | | | | | | | |
| Muchamiel | 0.090 a | 0.08 a | 0.046 a | −1.10 a | 0.339 a | 0.81 a | 0.038 a | 1.02 a | 0.202 b | 0.65 b |
| De la Pera | 0.075 a | −0.08 a | 0.033 ab | −1.26 b | 0.221 b | 0.63 b | 0.042 a | 1.06 a | 0.690 a | 1.21 b |
| Odissea | 0.077 a | 0.03 a | 0.016 b | −1.51 b | 0.235 b | 0.66 b | 0.034 a | 0.98 a | 0.309 b | 0.81 a |

[1]N.S. = not significant F ratio ($p < 0.05$); *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively.
[2]Values are the mean of three repetitions. Values followed by the same letter, within the same source of variation, are not significant different ($p < 0.05$).

**Table 5.** Odor descriptors of the studied volatile compounds (Baldwin et al., 2000; Saliba-Colombani et al., 2001).

| Aroma compound | Odor threshold (μL/L) | Odor descriptor |
|---|---|---|
| 3-Methylbutanal | 0.2 | Alcohol, stale |
| 1-Penten-3-one | 1 | Chemical, green note, plastic |
| Hexanal | 4.5 | Fatty, green, green tomato, herbaceous, grassy, powerful, penetrating |
| *cis*-3-Hexenal | 0.25 | Tomato-like, grass |
| *trans*-2-Hexenal | 17 | Sweet, fragrant, almond, fruity, green, leafy, apple, plum, vegetable |
| *cis*-3-Hexenol | 70 | Fresh, green, green grass |
| 1-Hexanol | 500 | Mint, grass |
| 6-Methyl-5-hepten-2-one | 50 | Herbaceous, green, oily, pungent, parsley, green bean |
| 2-Isobutylthiazole | 3.5 | Tomato leaves, fermented, plastic |
| Methyl salicylate | 40 | – |

Veit-Köhler et al. (1999) found positive relationships among aroma volatiles and sensory attributes in tomatoes. For instance, the hexanal odor has been described as 'green, herbaceous' (Table 5). In three seasonal studies, sweetness intensity was related to hexanal, with contributions from *cis*-3-hexenal, *trans*-2-hexenal, or *cis*-3-hexenol (Baldwin et al., 2000). Therefore, it could be inferred that *De la Pera* and *Muchamiel* tomatoes have a stronger 'green, herbaceous' odor/aroma than *Odissea* tomatoes.

The fact that *Muchamiel* and *De la Pera* types presented simultaneously the highest values in the sensory tests and the highest contents of most of the volatiles is a suggestive result. In this sense, Baldwin et al. (1998) found that tomato-like flavor intensity ratings for seven cultivars were almost identical to the rating for overall acceptability, indicating the close relationship between flavor and tomato quality. This is, however, not enough proof that these would be the most accepted tomato types by consumers because both the absolute concentrations of sugars and organic acids and the balanced ratio between them are also important factors in consumer acceptance (Hobson and Bedford, 1989; Malundo et al., 1995; Petersen et al., 1998). Increasing total sugar and organic acid levels of fresh tomato improved flavor acceptability (Malundo et al., 1995; Petersen et al., 1998), and a balanced sugar/organic acid ratio was preferred by a panel examining the flavor characteristics of cherry tomato (Petersen et al., 1998).

Poor flavor quality in tomato appears to be in part a result of breeding practices that do not select for flavor, because of lack of information (Baldwin et al., 2000). Sensory parameters that could assist the breeders in an efficient selection for flavor have not been characterized. The definition and use of markers that correlate to tomato flavor could improve this situation and provide the breeder and processor with analytical tools for flavor enhancement (Bucheli et al., 1999). Breeders could also use sensory analysis, but this is often difficult to perform and requires access to a panel and considerable expertise (Baldwin et al., 2000). Quantitative comparison of desirable flavor volatiles in addition to reducing sugars and free acids in tomato breeding lines would be a useful tool in selecting for a better tasting tomato.

Our results showed that the isolation technique for volatile compounds used is valid to detect quantitative differences among tomato types (traditional and modern ones) and has the advantage of using very low amounts of material; however, it has the disadvantage of being a destructive technique.

### Discriminant Analysis

The nontypified discriminant analysis (stepwise, discriminant analysis) was used to classify aroma profiles from different tomato types into three categories: (1) *Muchamiel*, (2) *De la Pera*, and (3) *Odissea*. Initially, the concentrations of 10 different volatile compounds were provided to the SPSS program (version 11.5); these compounds were: (1) 3-methylbutanal (3MB), (2) 1-penten-3-one (1P3O), (3) hexanal (HAA), (4) *cis*-3-hexenal (c3HEA), (5) *trans*-2-hexenal (t2HEA), (6) *cis*-3-hexenol (c3HEO), (7) 1-hexanol (1HAO), (8) 6-methyl-5-hepten (6M5H), (9) 2-isobutylthiazole (2IBT), and (10) methyl salicylate (MS).

The discriminant analysis showed that all 36 aroma profiles initially considered were valid and that the mathematical model could be built using only 6 out of the initial 10 volatiles provided (based on the values of Wilks Lambda and their significance); the six selected volatiles based on these are shown in Table 7. Table 6 summarizes the values of Wilks Lambda; since the values of the statistic were not very low (they were not close to zero), it was possible that the categories (*Muchamiel*, *De la Pera*, and *Odissea*) of the dependent variable (tomato type) were not completely discriminated. However, both the *p*-values and the statistics of the 'exact *F*' of the Wilks lambda certified the significance of the discriminant functions (DF); thus,

**Table 6.** Variables introduced/rejected in the stepwise discriminant analysis of tomato type.

| | | Wilks Lambda | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Exact F | | | |
| Step | Introduced variable | Statistic | df1 | df2 | df3 | Statistic | df1 | df2 | *p*-value |
| 1 | Methyl salicylate | 0.346 | 1 | 2 | 33 | 31.202 | 2 | 33 | <0.0001 |
| 2 | 1-Penten-3-one | 0.177 | 2 | 2 | 33 | 21.982 | 4 | 64 | <0.0001 |
| 3 | 3-Methylbutanal | 0.106 | 3 | 2 | 33 | 21.423 | 6 | 62 | <0.0001 |
| 4 | 6-Methyl-5-hepten-2-one | 0.068 | 4 | 2 | 33 | 21.339 | 8 | 60 | <0.0001 |
| 5 | 2-Isobutylthiazole | 0.045 | 5 | 2 | 33 | 21.579 | 10 | 58 | <0.0001 |
| 6 | *cis*-3-Hexenal | 0.033 | 6 | 2 | 33 | 21.122 | 12 | 56 | <0.0001 |

**Table 7.** Matrix structure of the discriminant analysis of tomato type.

| Volatile compound | Discriminant function 1 | Discriminant function 2 |
|---|---|---|
| 1-Penten-3-one | −0.431[1] | −0.119 |
| *cis*-3-Hexenol | 0.327[1] | 0.039 |
| 6-Methyl-5-hepten-2-one | 0.233[1] | −0.077 |
| 1-Hexanol | 0.226[1] | 0.050 |
| 3-Methylbutanal | 0.208[1] | 0.191 |
| Methyl salicylate | −0.398 | 0.516[1] |
| *cis*-3-Hexenal | 0.007 | 0.343[1] |
| *trans*-2-Hexenal[2] | −0.237 | 0.312[1] |
| Hexanal | 0.020 | −0.197[1] |
| 2-Isobutylthiazole | −0.019 | 0.145[1] |

[1]Major absolute correlation among each variable and any of the discriminant functions.
[2]This variable was not finally used in the analysis.

the discrimination among the categories of the tomato type will be fine.

The DF were as follows:

$$\begin{aligned} DF1 = {} & 233.416 \times 3MB - 63.644 \times 1P3O - 3.005 \\ & \times c3HEA + 9.624 \times 6M5H - 137.618 \\ & \times 2IBT - 0.230 \times MS + 2.367 \end{aligned}$$

$$\begin{aligned} DF2 = {} & 63.906 \times 3MB - 72.603 \times 1P3O + 2.852 \\ & \times c3HEA - 0.339 \times 6M5H - 13.967 \\ & \times 2IBT + 7.227 \times MS + 0.452 \end{aligned}$$

The first discriminant function (DF1) explained 75.7% of the variability of the model, while the DF2 explained the remaining 24.3%; both functions were significant according to the *p*-values of the Wilks lambda for the canonical DF, 0.000 and 0.004 for DF1 and DF2, respectively.

Table 7 shows that 1-penten-3-one, 6-methyl-5-hepten-2-one, 3-methylbutanal, *cis*-3-hexenol and 1-hexanol had a higher correlation with DF1 (however only the first three independent variables were selected for the final model, 1P3O, 6M5H, and 3MB) while the remaining five volatile compounds had a higher correlation with DF2 (only MS, c3HEA, and 2IBT were selected).

**Table 8.** Classification matrix of some traditional and hybrid Spanish tomatoes on the basis of their aroma profiles.

| | | Estimated tomato type | | |
|---|---|---|---|---|
| Tomato type | (%) Correct | MUCH | PER | ODI |
| MUCH | 100 | 21 | 0 | 0 |
| PER | 100 | 0 | 11 | 0 |
| ODI | 100 | 0 | 0 | 4 |

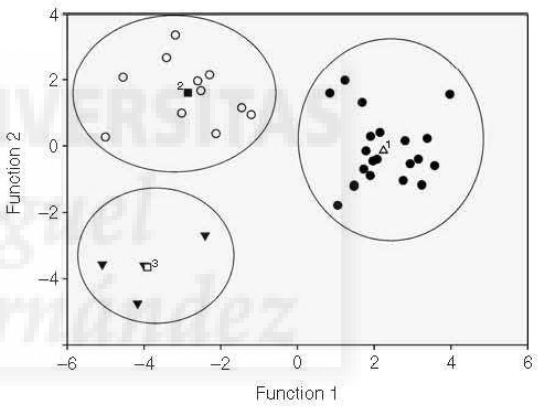


**Figure 1.** Scatter-plot for the traditional and hybrid Spanish tomatoes using the first two discriminant functions. (1), (2), and (3) group centroid. Tomatoes: (▼) hybrid; (○) de la Pera, (●) Muchamiel.

Table 8 summarizes the results of the discriminant classification of the 36 samples studied. All samples from the types *Muchamiel*, *De la Pera*, and *Odissea* were successfully discriminated. In summary, the present model successfully classified 100% of the studied samples (Figure 1).

Finally, three tomatoes were selected randomly out of the total 24 samples (8 tomatoes from each of the three tomatoe types), their aroma profiles were obtained and are shown in Table 9. These three aroma profiles were successfully discriminated as being of *De la Pera*, *Odissea*, and *Muchamiel*, respectively. In this validation,

**Table 9.** Concentrations of volatiles in three randomly selected tomatoes to validate the developed discriminant model.

| Volatile compound | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| 3-Methylbutanal | 0.038 | 0.027 | 0.045 |
| 1-Penten-3-one | 0.115 | 0.128 | 0.093 |
| Hexanal | 3.725 | 1.659 | 3.001 |
| *cis*-3-Hexenal | 0.975 | 0.629 | 0.868 |
| *trans*-2-Hexenal | 0.296 | 0.310 | 0.223 |
| *cis*-3-Hexenol | 0.075 | 0.077 | 0.090 |
| 1-Hexanol | 0.033 | 0.016 | 0.046 |
| 6-Methyl-5-hepten-2-one | 0.221 | 0.235 | 0.339 |
| 2-Isobutylthiazole | 0.042 | 0.034 | 0.038 |
| Methyl salicylate | 0.690 | 0.309 | 0.202 |
| Original type | PER | ODI | MUCH |
| Estimated type | PER | ODI | MUCH |

the model was able to correctly assign 100% of the samples.

## CONCLUSIONS

Tomato breeders are nowadays considering tomato flavor as one of the key parameters of fruit quality because consumers care more and more for sensory issues. Methods to analyze volatile compounds that need low amounts of tomato samples are needed to carry out selection of individual genotypes. As analysis of flavor compounds in the aromatic component requires expensive equipment and training, if volatile determination is going to be used as a tool in selection programs, a low number of samples should be needed. Tomato aroma is complex, more than 400 volatiles have been identified and a combination of about 20 compounds gives tomato its unique odor characteristics. However, reducing the number of compounds to a few ones (<10) with major contributions to aroma could increase the usefulness of volatile determinations in tomato breeding programs. Significant differences among traditional and hybrid tomato types were found by the purge-and-trap technology and a mathematical model that successfully discriminated among tomato types was developed using only the concentrations of six volatile compounds: 3-methylbutanal, 1-penten-3-one, hexanal, *trans*-2-hexenal, 1-hexanol, and 2-isobutylthiazole.

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ORIGINAL PAPER

# Volatile compounds of traditional and virus-resistant breeding lines of *Muchamiel* tomatoes

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**Abstract** Currently, sensory quality is the primary objective for almost all tomato breeding programs. In this study, postharvest behaviour of a breeding line with genetic resistance to important viruses (ToMV, TSWV, and TYLCV) has been compared with the original traditional landrace (*Muchamiel*) from an aromatic point of view. The breeding line has been obtained by backcrossing, introgressing three resistance genes but trying to keep the aromatic characteristics of the traditional variety. The main aim was to obtain qualitative (solid phase microextraction, SPME) and semi-quantitative (simultaneous steam-distillation extraction, SDE, and hydrodistillation, HD) volatile compositions of *Muchamiel* tomatoes. Fruits were randomly picked at a green-immature stage and stored at 10 °C during 13 days. Volatile compositions of tomatoes in two stages of ripening (green-immature and red-mature) were obtained by three different extraction methods: SDE, HD, and SPME. Besides, sensory evaluation with a trained panel was carried out on both types of tomatoes at two ripening stages. Main fresh tomato aroma compounds (*cis*-3-hexenal, hexanal, *cis*-3-hexanol, 6-methyl-5-hepten-2-one, 2-isobutylthiazole and $\beta$-ionone) were found at similar concentrations in both types of samples, confirming the success of the breeding program. Finally, SPME could be considered as a useful tool to get realistic values on fresh tomato odour, while HD and SDE results correlated better with fresh tomato aroma. Although a long time has been required to develop the breeding line, results indicate that sensory quality has been recovered through backcrossing, confirming the success of the breeding program.

**Keywords** Aroma · HD · Genetic improvement · SDE · *Solanum lycopersicum* · SPME

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## Introduction

Until few years ago, tomato breeders mainly concentrated in improving yield, fruit size, fruit appearance (attractive red colour and lack of defects: spots, cracks, etc.), disease resistance and, more recently, fruit firmness and shelf life (to allow long-distance trading). Sometimes, if a consumer closes its eyes while eating a "modern greenhouse" tomato, it could taste as any other juicy and fresh fruit. However, and according to a market survey [1] based on consumers' preference, the sensory quality of a food product has been ranked higher than its nutritional value, price or safety.

After its arrival to Spain, tomato began a process of diversification and adaptation to the different agro-climatic conditions of different Spanish regions. During this period, special attention was paid to organoleptic quality, and a great array of traditional tomato cultivars were originated [2]. Several traditional cultivars or landraces still survive in the orchards of Southern and Eastern Spain. Frequently ignored outside their production area, all of them are highly esteemed by local people due to their excellent sensory quality and are much more expensive than hybrid varieties. These landraces are highly susceptible to several viruses, such as the ToMV, TSWV, and TYLCV [3], the incidence

of which makes their cultivation non-viable. As a consequence of a breeding program for the introduction of genetic resistance (genes *Tm-2*$^a$, *Sw-5*, and *Ty-1*) into traditional tomato cultivars, we have developed several breeding lines corresponding to the *Muchamiel* and *De la Pera* types (Valencian Community, Eastern Spain).

Organoleptic quality involves odour, taste and aroma (flavour), colour and texture of fruit. Flavour depends, among other factors, on sugar and acid contents, the sugar/acid ratio but also on the presence and concentration of different characteristic volatile compounds. Breeding for organoleptic quality has been severely restricted by the lack of efficient criteria and by the polygenic nature of the trait.

The influence of variety, ripening stage, and storage conditions on composition of tomato odour and aroma was proved, but little is known about their genetic control [4]. In previous studies [5, 6], it was demonstrated that the traditional types of Spanish tomatoes (cv. *Muchamiel* and cv. *De la Pera*) presented higher intensities of tomato odour and aroma according to a trained panel and were more accepted by a consumer panel than hybrid samples (cv. *Odissea*). Besides, the traditional tomatoes showed significantly higher concentrations of several tomato-impact compounds, such as 3-methylbutanal, hexanal, *cis*-3-hexenal, 1-hexanol and 2-isobutylthiazole. Determination of the tomatoes' volatile compounds could be a useful tool to understand consumer acceptance and should be included in postharvest studies.

Simultaneous steam-distillation extraction (SDE) provides good results for aroma extraction in several matrices and recovery percentages of compounds were in the range 80–108% [7]. However, the main drawback of SDE is the formation of artefacts mainly due to intense heating and long extraction times. It is not probably the best technique to obtain aroma profiles of fresh fruits but could be a very good tool to quantify those compounds previously pointed as impact-chemical by other techniques. Hydrodistillation (HD), using Deryng apparatus, is a technique mainly used in the extraction of essential oils from fresh fruits [8–10]. The heating in HD is less intense than in SDE and a concentration step with low control and potential losses of volatile compounds is avoided because only 1 mL of organic solvent is used. The third extraction method under study was solid phase microextraction (SPME), which is a well-known and widely used technique in food. It is a simple, fast and solvent-less sample preparation technique that can perform sampling, clean-up and concentration in only one step [11]. SPME is a non-quantitative extraction technique, but the heating of the sample is minimum and the volatile composition obtained should be closer to real one than those obtained by heating techniques such as SDE and HD.

The global aim of this study was to demonstrate that genetic improvement for disease resistance did not significantly reduce the aroma quality of *Muchamiel* tomatoes due to the consideration of the main tomato sensory properties during the breeding program. With this general aim in mind, the specific objective was to compare three extraction techniques for the isolation of the tomato extracts: (1) SDE, (2) HD, and (3) SPME at two ripening stages: (a) green-immature and (b) red-mature tomatoes. Results should help to determine the best way to obtain realistic qualitative and/or quantitative tomato volatile compositions and therefore be of help to breeders, farmers, and people involved in commercializing tomatoes to understand that genetic improvement for disease resistance could be achieved without reducing sensory quality.

## Materials and methods

### Chemicals

All the aroma standards (2-pentanol, 3-methyl-2-butanol, *cis*-3-hexenal, hexanal, *cis*-2-hexenal, *trans*-2-hexenal, *cis*-3-hexenol, hexanal, α-pinene, *trans*-2-heptenal, 6-methyl-5-hepten-2-one, hexanoic acid, *p*-cymene, limonene, eucalyptol, 2-isobutylthiazole, *trans*-2-octenal, linalool oxide, linalool, guaiacol, nonanal, 2-phenylethanol, camphor, *trans*-2-nonenal, methyl salicylate, α-terpineol, decanal, citral (neral and geranial), geraniol, *trans*-2-decenal, *trans*–*trans*-2,4-decadienal, eugenol, damascenone, and β-ionone) used for identification and quantification purposes were obtained from Sigma-Aldrich (Madrid, Spain).

### Plant material and growing conditions

Plants of a traditional *Muchamiel* landrace and a *Muchamiel* breeding line with genetic resistance to ToMV, TSWV, and TYLCV were grown in the greenhouses of an organic farm in Lorca, Murcia (Eastern Spain), over a spring–summer growing cycle. Tomatoes were harvested on 3 June 2008. Fruits of *Muchamiel* cultivars are large in size (>200 g), flattened and strongly ribbed. Forty-day-old seedlings were transplanted at the beginning of March. Plants were allowed to grow vertically with a single stem. Pollination was improved using bumblebees (*Bombus terrestris*). Standard irrigation for tomato crop was used and only organic matter was applied before transplanting to maintain the soil fertility.

### Sampling and samples processing

Tomatoes were picked at the green-immature stage of ripening, selected according to the USDA standard tomato colour classification chart [12]. One hundred units of each type of fruit (traditional and virus-resistant tomatoes) at the

same stage of ripening were randomly harvested from different plants of an organic farm. Once in the laboratory, 50 fruits of each tomato type were grinded, mixed with $CaCl_2$ (150 mL $kg^{-1}$ tomato), and stored in individual bags (100 g of tomato per bag) at −80 °C until the analysis time. The rest of the fruits (50 units per tomato type) were stored at 10 °C and 90% relative humidity during 13 days until the tomatoes were in a red-mature stage of ripening and then they were treated as previously described for green-immature fruits.

Extraction procedure of volatile aroma compounds

*Simultaneous steam-distillation extraction*

Simultaneous steam-distillation extraction was used for volatile isolation in tomatoes at two different stages: (1) day 0 (green-immature stage) and (2) day 13 (red-mature stage). A suspension of 75 g of tomato samples in 150 mL of ultrapure water (Milli-Q, Millipore Corp. Bedford, MA, USA) was placed in the sample flask (A) of the Likens-Nickerson distillator (Afora, Barcelona, Spain), with 15 g NaCl (Merck, Darmstadt, Germany) and boiling chips. In the organic solvent flask (B), 50 mL of dichloromethane, $Cl_2CH_2$ (Labscan Ltd, Dublin, Ireland), was introduced together with boiling chips. 100 µL of 2-methylpyrazine, 1 g $L^{-1}$, was added as internal standard; the experimental recovery percentage for 2-methylpyrazine in an aqueous matrix in SDE was 108%. Flask B was heated in a water bath at 55 °C and flask A in an oil heater at 160 °C. The vapours were condensed by means of a cold refrigerant maintained at −5 °C by a cryostat, model Frigiterm (Selecta, Barcelona, Spain). After 120 min of extraction, the organic solvent contained the aroma compounds from tomato samples [13, 14]. The extract was dried over 5 g anhydrous $Na_2SO_4$ (Panreac Química S.A., Barcelona, Spain), concentrated to about 1 mL with a Vigreux column and finally to 0.4 mL under a $N_2$ stream. SDE experiments were run, at least, in triplicate.

*Hydrodistillation*

Hydrodistillation using a Deryng apparatus was also used for aroma extraction of tomatoes. A suspension of 100 g of tomato samples was placed in the sample flask together with 50 mL of ultrapure water, 20 g NaCl and 100 µL of 2-methylpyrazine, 1 g $L^{-1}$, as internal standard. Sample flask was heated until boiling during 1 h. The vapours were condensed by means of a cold refrigerant maintained at −5 °C by a cryostat, model Frigiterm (Selecta). After 60 min of extraction, the solvent, 1 mL of pentane (Panreac), containing the aroma compounds, was collected.

*Solid phase microextraction*

Solid phase microextraction was the third extraction method under study. After several preliminary tests to optimize the extraction system, 4 mL of ground sample with 3 mL of distillate water was hermetically placed in a 15 mL vial with a polypropylene hole cap PTFE/silicone septa. A small magnetic stirring bar was added and the vial was placed in a water bath with temperature control and stirring. Vials were equilibrated during 5 min at 40 °C in the bath and after this equilibration time, a 50/30 µm DVB/CAR/PDMS fibre was exposed to the sample headspace for 60 min at 40 °C. The fibre was chosen for its high capacity of trapping fruits volatile compounds [15] and a similar extraction procedure was previously carried out in tomatoes by Serrano [11] and Markovic et al. [16].

After sampling, the desorption of the volatile compounds from the fibre coating was carried out in the injection port of the GC at 250 °C during 1 min in split mode.

Chromatographic analyses

The isolation, identification, and quantification of the volatile compounds were performed on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GCMS QP-5050A. The GC–MS system was equipped with a TRACSIL Meta.×5 column (Teknokroma S. Coop. C. Ltd, Barcelona, Spain; 30 m × 0.25 mm × 0.25 µm film thickness). Analyses were carried out using conditions previously described by Vázquez et al. [13] and Vázquez-Araújo et al. [7, 17].

Most of the compounds were identified using three different analytical methods: (1) Kovats indices, (2) GC–MS retention indices (authentic chemicals), and (3) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered tentative when it was based only on mass spectral data [18].

For the quantification of the volatile compounds, 2-methylpyrazine was added as internal standard in both SDE and HD; this chemical was used as internal standard after checking that it was absent in Spanish tomatoes and under the proposed conditions, it separates well from other volatile compounds. Data included in this study should be considered as semi-quantitative, because no standard curves were carried out for each one of the quantified volatile compounds. However, relative values are useful to compare differences between tomato lines.

All the aroma standards used for identification and quantification purposes were food grade (Sigma-Aldrich, Flavors and Fragrances, Milwaukee, WI, USA).

### Sensory evaluation with trained panel

Sensory evaluation with trained panel was used to discriminate the intensities of "fresh tomato" odour (perception of volatile compounds sniffed through the nose), aroma (perception of volatile compounds during chewing of samples) and other properties in both tomato cultivars such as: colour, hardness, sweetness, acidity, etc. Both tomato types (traditional and virus-resistant, VR) were studied at green-immature and red-mature ripening stages.

Tomatoes were tested by a panel of ten panelists, aged 20–50 years (6 female and 4 male, all members of the University Miguel Hernandez), with sensory evaluation experience and trained in descriptive evaluation of tomato.

The panel was selected and trained following the ISO standard 8586-1 [19]. For the selection of the panel members, and after rejecting candidates with obvious drawbacks, ranking/rating tests for intensity of acid, sweet, and tomato volatile compounds were carried out. These tests were used to determine candidates' ability to discriminate and describe graded levels of intensity of a given attribute. Series of samples in random order were presented to candidates, in which one parameter is present at different levels, which cover the range present in the products of interest. Candidates ranking samples correctly or inverting only adjacent pairs were selected; the same rank order criterion was used for the rating tests [19, 20].

Training sessions were carried out twice a week for 1 month, that is, ten training sessions of 2 h were needed (20 h). The amount of time needed depends on the complexity of the product, on the experience of the panelists and their knowledge on the product, on the number of attributes to be covered, and on the requirements for validity and reliability [20]. All panelists selected had more than 1 year's experience in discrimination and descriptive test on a variety of foods. Further details about panel selection and training could be found in Ruíz et al. [21].

Measurements were performed in individual booths with controlled illumination and temperature [19, 20].

The individual products were scored for the intensity of different sensory parameters (colour, odour, sweetness, acidity, aroma, hardness, and juiciness) on a scale of 0–10, where:

- 0 = extremely low intensity.
- 5 = regular intensity in fresh tomatoes.
- 10 = extremely high intensity.

Panelists relied on their training experience to score products and tomatoes were presented as fresh wedges in 50 mL plastic beakers with lids.

### Statistical analyses

Data from tomatoes' analyses were examined by analysis of variance (ANOVA) using STATGRAPHICS Plus 5.0 software (Manugistics, Inc., Rockville, MD). Wherever $F$ values were significant, Tukey's multiple-range test was used to separate the mean effects. Significance was defined at $P \leq 0.05$. Graphics were done using Sigma Plot 9.0 (SPSS Science, Chicago, IL, USA).

## Results and discussion

### Sensory analysis

Flavour quality of fruits and vegetables is influenced by genetic, pre-harvest, harvesting and postharvest factors. The postharvest life based on flavour and nutritional quality is usually shorter than that based on appearance and textural quality. It is essential to obtain high-quality tomatoes by selecting the best-tasting genotypes in breeding programmes [22]. Consequently, several studies have been carried out about the sensory quality of tomatoes [5, 22–24].

#### *Green-immature stage*

At this ripening phase, virus-resistant (VR) tomatoes showed higher sweetness (6.6 compared to 4.7) and lower acidity (4.3 compared to 6.3) than traditional fruits (Table 1). On the other hand, VR tomatoes presented significantly lower intensity of tomato odour than traditional fruits (5.2 compared to 6.2, respectively). Hardness scores were higher in VR tomatoes than in the original landrace (6.5 compared to 4.7), which will be an advantage during postharvest operations, including washing, calibration, cold storage, distribution, etc. There were no significant differences in the perception of the fresh tomato aroma, with a mean value for both traditional and VR fruits being 6.0 (Table 1). Finally, the juiciness was not influenced at all by the genetic improvement of *Muchamiel* tomatoes (ranged from 6.6 to 5.6; mean of 6.5). In summary, genetic improvement of *Muchamiel* tomatoes resulted in immature-green samples with many advantages (high intensities of sweetness and hardness and low acidity) and the only drawback of having slightly, but significant, lower fresh tomato odour than traditional fruits.

#### *Red-mature stage*

Considering the fact that the general aim of this study was to demonstrate that the genetic improvement carried out did not reduce the quality of *Muchamiel* tomatoes, fresh tomato

**Table 1** Sensory scores gave for the trained panel of traditional and virus-resistant (VR) *Muchamiel* tomatoes at green-immature and red-mature stage of ripening

| Ripening stage | Sensory scores | | | | | | |
|---|---|---|---|---|---|---|---|
| | Fresh tomato odour | Sweetness | Acidity | Fresh tomato aroma | Hardness | | Juiciness |
| | | | | | Skin | Pulp | |
| Green-immature | | | | | | | |
| Traditional tomatoes | 6.7 ± 0.6 a | 4.7 ± 0.5 b | 6.3 ± 0.6 a | 6.3 ± 0.7 | 5.8 ± 0.5 | 4.7 ± 0.6 b | 6.6 ± 0.5 |
| VR tomatoes | 5.2 ± 0.5 b | 6.6 ± 0.6 a | 4.3 ± 0.4 b | 5.7 ± 0.6 | 6.6 ± 0.6 | 6.5 ± 0.5 a | 6.3 ± 0.3 |
| Red-mature | | | | | | | |
| Traditional tomatoes | 5.1 ± 0.7 | 4.6 ± 0.5 | 5.0 ± 1.1 a | 4.9 ± 1.0 | 5.4 ± 0.7 | 5.4 ± 0.6 | 6.0 ± 0.8 |
| VR tomatoes | 6.3 ± 0.6 | 5.6 ± 0.6 | 4.7 ± 0.8 b | 5.7 ± 0.7 | 6.4 ± 0.9 | 6.3 ± 0.4 | 5.6 ± 0.6 |

Values followed by the different letter, in the same column and ripening stage, were significantly different ($P < 0.05$), Tukey's multiple-range test

aroma and odour and hardness could be considered as the most relevant sensory attributes. As can be seen in Table 1, the differences among the intensities of these three main sensory attributes in the traditional and VR tomatoes were not statistically significant. This stage of ripening is quite close to the marketing phase and it can be considered as the most important of the two ripening stages under study. Although no statistical significant differences were found, VR tomatoes always had higher values of hardness in both skin and pulp than traditional fruits. These experimental results indicate that the process of genetic improvement for virus resistance of the *Muchamiel* landrace has resulted in tomatoes with intense fresh tomato odour and aroma and of ideal hardness at the commercial ripening stage.

## Volatile compounds

Tieman et al. [25] identified 25 loci that influence the chemical composition of ripe tomatoes, and significantly altered the contents of at least one volatile compound. These authors concluded that linked molecular markers should be useful for breeding programmes aimed at improving fruit flavour. However, our goal was different, proving that breeding for virus-resistant lines could also be done without losing aromatic quality.

A total of 39 volatile compounds were found in the different extracts (SDE, HD, and SPME) of traditional and VR *Muchamiel* tomatoes (Table 2). Buttery [26] reported that fresh tomato aroma could be closely duplicated with only ten compounds: *cis*-3-hexenal, *cis*-3-hexanol, hexanal, 1-penten-3-one, 3-methylbutanal, *cis*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and *β*-ionone. Six out of these ten compounds were detected in the three extraction methods under study: hexanal, *cis*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and *β*-ionone. Besides, *cis*-3-hexenal was detected in SDE and HD. These experimental results supported the importance of the volatile compounds included in Buttery's list [26].

Birtić et al. [27] proved again that most of these compounds contribute to the tomato aroma after studying the volatile compounds of Cervil and Levovil mature tomatoes. These authors obtained positive log odour units for 26 volatiles, including *cis*-3-hexenal, hexanal, *cis*,*cis*-2,4-decadienal, *β*-ionone, *cis*-2-hexenal, 3-methylbutanol, and 2-isobuthylthiazole; besides, the important role of eugenol, 2-methoxyphenol and 2-methylthioacetaldehyde was also reported. Mayer et al. [28] studied the flavour profiles of three tasty and two less tasty fresh greenhouse tomato cultivars. Compounds such as 1-pente-3-one, (*E*,*E*)- and (*Z*,*Z*)-2,4-decadienal, and furaneol had higher odour units the more preferred cultivars, whereas methional, phenylactaldehyde, 2-phenylethanol, or 2-isobutylthiazole had higher odour units in the less preferred cultivars.

Buttery and Takeoka [29] reported that there were some minor volatiles such as 5-ethyl-2(5H)-furanone or 5-ethyl-cyclopentene-1-carbaldehyde, not important for the global aroma, but indicative of aroma degradation. Concina et al. [30], using dynamic-headspace GC–MS, found significant differences in the semi-quantitative volatile compositions of fresh and spoiled tomato samples. For instance, samples contaminated by *E. coli* showed higher contents of ethyl acetate and 6-methyl-5-hepten-2-one. Similar studies were carried out by Bianchi et al. [31], who concluded that five volatiles, i.e. ethanol, *β*-myrcene, *o*-methyl styrene, 6-methyl-5-hepten-2-ol and 1-octanol, were found to be able to better discriminate between uncontaminated and contaminated samples. None of these five compounds were found in the present experiment proving the low pollution level and proper quality of the tomatoes under study.

Abegaz et al. [32] develop predictive models of sensory descriptors as a function of volatile and non-volatile compounds. These models from taste followed by aroma supported the important role of ethanol to the perception of

**Table 2** Volatile compounds found in traditional and virus-resistant (VR) *Muchamiel* tomatoes

| Compound | Kovats index | | O.T. ($\mu g\ L^{-1}$)[A] | Extraction method | Descriptor | Literature |
|---|---|---|---|---|---|---|
| | Experimental | Literature | | | | |
| 2-Pentanol | 733 | 717 | 4,000[a] | SPME | Oily, green | [34] |
| 3-Methyl-2-butanol | 755 | | 71[b] | SDE | Pungent, earthy | [17, 34, 36–41] |
| *cis*-3-Hexenal | 798 | 800 | 26[b] | SDE, HD | Tomato leaf-like | [11, 17, 34–38] |
| Hexanal | 798 | 801 | 479[b] | SPME, SDE, HD | Fatty, green, grass | [5, 11, 34, 36–38] |
| *cis*-2-Hexenal | 840 | 854 | | SDE, HD | | |
| *trans*-2-Hexenal | 848 | 854 | 123[b] | SPME, SDE, HD | Almond, fruity, green, apple | [5, 17, 34–38] |
| *cis*-3-Hexenol | 854 | 858 | 347[b] | SDE, HD | Fresh, green grass | [5, 17, 34–37] |
| Hexanol | 874 | 869 | 759[b] | SDE, HD | | [5, 17, 34–37] |
| α-Pinene | 927 | 930 | 6[a] | SPME, HD | Woody | [35, 38] |
| *trans*-2-Heptenal | 954 | 956 | 60[c] | SPME, SDE, HD | Apple, lemon, green, vegetable | [34, 35, 37] |
| 6-Methyl-5-hepten-2-one | 985 | 994 | 525[b] | SPME, SDE, HD | Herbaceous, green, oily | [11, 17, 34, 35, 37] |
| Hexanoic acid | 1012 | 1020 | 3,000[d] | SDE | Cheesy, fatty, sour | [17] |
| *p*-Cymene | 1022 | 1024 | 11.4[d] | SPME, SDE, HD | Citrus | [38] |
| Limonene | 1026 | 1031 | 10[d] | SPME, SDE, HD | (+) Lemon, orange, citrus (−) Herbaceous, minty | [11, 35] |
| Eucalyptol | 1027 | 1033 | 1.3[d] | HD | Citrus, herbaceous, fruity | |
| 2-Isobutylthiazole | 1031 | 1043 | 29[b] | SPME, SDE, HD | Tomato leaves | [5, 11, 17, 34–37] |
| *trans*-2-Octenal | 1058 | 1060 | 3[d] | SPME, SDE, HD | Green, herbaceous, spicy | [35] |
| Linalool oxide | 1072 | 1078 | 320[d] | SDE, HD | | |
| Linalool | 1103 | 1101 | 6[d] | HD | Lemon, orange, citrus, floral | [11, 17, 34, 35, 37] |
| Guaiacol | 1083 | 1095 | 3[e] | SDE | Medicinal, smoky, woody | [11, 35, 38] |
| Nonanal | 1105 | 1102 | 1[d] | SPME, SDE, HD | Apple, coconut, grape, lemon | [35, 38] |
| 2-Phenylethanol | 1117 | 1120 | 479[b] | SDE | Honey, floral, rose | [11, 17, 34–37] |
| Camphor | 1143 | 1143 | 460[d] | HD | Medicinal, woody, vanilla | [35] |
| *trans*-2-Nonenal | 1162 | 1159 | 0.08[d] | SDE, HD | Waxy, fatty | [38] |
| Methyl salicylate | 1197 | 1198 | 40[a] | SPME, SDE, HD | Minty, spicy, sweet | [11, 34, 37, 38] |
| α-Terpineol | 1198 | 1192 | 350[d] | SDE | Lilac | [11, 35] |
| Decanal | 1209 | 1207 | 0.1[d] | SPME, SDE, HD | Floral, citrus, sweet | [38] |
| β-Cyclocitral[B] | 1222 | 1224 | 2[c] | SPME, SDE, HD | Geranium, rose | [11, 34, 38] |
| Neral | 1245 | 1240 | 3[d] | SPME, SDE, HD | Lemon | [11, 35, 37] |
| Geraniol | 1262 | 1259 | 40[d] | HD | Fruity, rose, sweet | [17] |
| *trans*-2-Decenal | 1266 | 1266 | 0.4[d] | HD | Oily, citrus, floral, green | |
| Geranial | 1275 | 1270 | 32[d] | SPME, SDE, HD | Lemon | [17, 34] |
| *cis,cis*-2,4-Decadienal[B] | 1300 | 1270 | | SDE | | |
| *trans–trans*-2,4-Decadienal | 1324 | 1312 | 0.07[e] | SDE | Citrus, fatty, meaty | [11, 35, 37] |
| Eugenol | 1363 | 1356 | 6[d] | SPME, HD | Cinnamon, clove, spicy | [17, 35, 38] |
| Damascenone | 1387 | 1383 | 10[d] | HD | Apple, woody, herbaceous | [34] |
| Geranyl acetone[B] | 1460 | 1452 | 186[b] | SPME, SDE, HD | Lavender, fruity, rose, sweet | [11, 34–37] |
| β-Ionone | 1493 | 1422 | 23[b] | SPME, SDE, HD | Warm, woody, floral | [5, 11, 34–37] |
| Pseudoionone[B] | 1589 | 1581 | 10[c] | HD | | [34] |

[A] References for the odour thresholds: [a] [39]; [b] [36]; [c] [34]; [d] [40]; [e] [41]

[B] Compounds tentatively identified; authentic standards were used for identification of all other compounds included in this table

sweetness, 2 + 3-methylbutanol, 6-methyl-5-hepten-2-one and geranylacetone to tomato-like, and hexanal to astringency.

The instrumental section of this experiment had three main objectives: (1) to compare the volatile compositions of traditional and VR *Muchamiel* tomatoes at two ripening

stages using three different extracting techniques (SDE, HD, and SPME), (2) to confirm the sensory results, which suggested that genetic improvement of *Muchamiel* tomatoes did not decrease the aromatic quality of these fruits, and (3) to look for the presence of off-flavours originated during the breeding program.

Solid phase microextraction, a qualitative technique mainly representative of foods odour, was carried out first. SPME and odour are easily correlated because both concepts consider the concentration and perception of the volatiles in the headspace of tomatoes. There were no important qualitative differences in the composition of SPME extracts of traditional and VR *Muchamiel* tomatoes at any of the two studied ripening stages. Only one of the compounds mentioned by Buttery [26], 2-isobutylthiazole (2-IBT), was present in VR tomatoes and was not found in traditional samples (Table 3). The sensory descriptor of 2-isobutylthiazole is "tomato leaves" (Table 2), and therefore the exclusive presence of 2-IBT in VR *Muchamiel* tomatoes was a positive sign of their good aromatic quality. The other differences between traditional and VR *Muchamiel* lines were detected in compounds which represented less than 1% of the total abundance of volatile compounds; these compounds included α-pinene, *p*-cymene, neral (*Z*-citral), and β-ionone. For instance at the red-mature stage, *p*-cymene, neral, geranyl acetone, and β-ionone were only present in VR tomatoes while only α-pinene was exclusively found in the traditional fruits.

In general, hexanal was the main compound in traditional tomatoes, with abundance percentages in the headspace of around 40% in both green-immature and red-mature tomatoes; while 6-methyl-5-hepten-2-one was the main compound found in VR tomatoes, with percentages of 44 and 66% in green-immature and red-mature tomatoes, respectively. These two compounds have similar odour descriptors: fatty, green, grassy, herbaceous, and oily. The added abundance percentages of these two compounds did not positively correlate with the odour scores from the trained panel. However, the added abundance percentages of the six compounds from Buttery's list [26] found in the SPME extracts of *Muchamiel* tomatoes were 85.8% (green-traditional), 83.6% (green-VR), 81.7% (red-traditional), and 87.6% (red-VR) tomatoes. These abundance percentages showed a positive correlation with the odour scores

**Table 3** Volatile compounds found in SPME extracts of traditional and virus-resistant (VR) *Muchamiel* tomatoes

| Compound | Abundance (%) | | | |
|---|---|---|---|---|
| | Green-immature stage | | Red-mature stage | |
| | Traditional | VR | Traditional | VR |
| 2-Pentanol | 9.72 ± 1.22 a | 7.69 ± 0.24 b | 5.10 ± 0.16 a | 0.90 ± 0.13 b |
| Hexanal | 41.5 ± 1.4 a | 22.4 ± 0.8 b | 38.4 ± 0.9 a | 19.7 ± 0.2 b |
| *trans*-2-Hexenal | n.d. | n.d. | n.d. | 3.56 ± 0.38 |
| α-Pinene | n.d. | n.d. | 0.67 ± 0.02 | n.d. |
| *trans*-2-Heptenal | 1.23 ± 0.24 | 1.12 ± 0.03 | n.d. | n.d. |
| 6-Methyl-5-hepten-2-one | 17.3 ± 0.7 b | 43.5 ± 1.5 a | 31.9 ± 2.8 b | 65.8 ± 1.0 a |
| *p*-Cymene | n.d. | n.d. | n.d. | 0.40 ± 0.01 |
| Limonene | 0.80 ± 0.04 b | 1.33 ± 0.13 a | 8.91 ± 0.25 a | 0.77 ± 0.08 b |
| 2-Isobutylthiazole | n.d. | 5.57 ± 0.12 | n.d. | 2.51 ± 0.04 |
| *trans*-2-Octenal | 2.89 ± 0.40 a | 1.85 ± 0.16 b | 2.59 ± 0.38 a | 0.71 ± 0.04 b |
| Nonanal | 6.54 ± 0.51 | 6.25 ± 0.14 | 6.33 ± 0.69 a | 1.20 ± 0.25 b |
| Methyl salicylate | 10.7 ± 0.7 a | 3.74 ± 0.30 b | n.d. | n.d. |
| Decanal | 3.76 ± 0.33 | 3.13 ± 0.40 | 3.68 ± 0.60 a | 0.37 ± 0.09 b |
| β-Cyclocitral | n.d. | n.d. | 1.15 ± 0.01 a | 0.90 ± 0.01 b |
| Citral | n.d. | n.d. | n.d. | 0.90 ± 0.02 |
| Geranial | 0.45 ± 0.13 a | 1.11 ± 0.18 a | 1.30 ± 0.65 | 1.71 ± 0.01 |
| Eugenol | 4.01 ± 2.51 | 1.36 ± 1.09 | n.d. | n.d. |
| Geranyl acetone | 1.05 ± 0.21 | 0.94 ± 0.33 | n.d. | 0.31 ± 0.02 |
| β-Ionone | n.d. | n.d. | n.d. | 0.30 ± 0.02 |

Values are the mean of at least three replicates

Values followed by the different letter, in the same row and ripening stage, were significant different ($P < 0.05$), Tukey's multiple-range test

*n.d.* not detected

from the trained panel ($R^2 = 0.7156$), demonstrating that SPME could be considered a useful analytical method to get realistic values about tomato odour.

With the aim of obtaining semi-quantitative (quantification was only based on the use of an internal standard) volatile compositions, HD and simultaneous extraction–distillation (SED) were also carried out. The extracts obtained using these two isolation procedures contained up to 39 compounds (Table 2); however, only the 13 compounds more widely reported in specialized studies were quantified in this particular experiment and their concentrations are reported in Table 4. These techniques have the drawback that the samples must be heated at approximately 100 °C for 1 h to obtain a representative extract and some artefacts as furfural can be generated [17]. However, the isolation procedure is quite complete and almost all volatile compounds initially present in the sample will be fully extracted.

Birtić et al. [27] found that the compound with the highest contribution to the aroma of tomatoes was *cis*-3-hexenal, which has an aroma descriptor to apple, grape, lilac, orange, or leaf-like. This compound was mainly found in SDE and HD extracts, proving its low volatility, and the fact that SDE and HD techniques could also be useful in the study of tomato flavour, because, for instance, the oxidation of *cis*-3-hexenal leads to 5-ethyl-2(5*H*)-furanone, which is mainly found in tomato plant leaves [29]. Another fact proving the goodness of SDE was that 2-phenylethanol was found only in SDE extracts. This compound is important for the aroma and flavour of many foods, including ripe tomato fruits [33].

Our working hypothesis was that HD and SED extracts will correlate better with aroma scores than with odour scores. In fact, the total concentrations of volatile compounds obtained using both extraction techniques agreed better with the intensities of tomato aroma than with those of tomato odour. The coefficient of determination ($R^2$) were higher for the aroma scores (aroma-HD $R^2 = 0.6595$ and aroma-SDE $R^2 = 0.3907$) than for odour scores (odour-HD $R^2 = 0.3001$ and odour-SDE $R^2 = 0.0017$). Even though the total concentrations of volatiles were always higher in the SDE than in the HD extracts, results from the HD technique showed a better positive correlation with the sensory data than those of SDE, perhaps because heating is too intense in this last technique.

## Conclusions

A long time has been required to introgress three resistance genes into a *Muchamiel* traditional landrace. However, the comparison of the sensory profiles and volatile composition of traditional and VR tomatoes during cold storage indicates that organoleptic fruit quality has been recovered through the backcrossing program. Even more, fruits of the breeding line demonstrated a better postharvest behaviour

**Table 4** Concentrations of volatile compounds (mean of, at least, three replicates) found in HD and SDE extracts of traditional and virus-resistant (VR) *Muchamiel* tomatoes

| Compound | SDE (mg kg$^{-1}$) | | | | HD (mg kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Green-immature stage | | Red-mature stage | | Green-immature stage | | Red-mature stage | |
| | Traditional | VR | Traditional | VR | Traditional | VR | Traditional | VR |
| 3-Methyl-2-butanol | 0.79 ± 0.25 | 0.52 ± 0.28 | 0.30 ± 0.12 | 0.22 ± 0.04 | n.d. | n.d. | n.d. | n.d. |
| *cis*-3-Hexenal | 0.62 ± 0.04 a | 0.36 ± 0.03 b | 0.66 ± 0.07 | 0.60 ± 0.06 | 0.03 ± 0.02 | 0.02 ± 0.01 | n.d. | n.d. |
| Hexanal | 0.87 ± 0.11 | 0.73 ± 0.04 | 1.30 ± 0.10 a | 0.95 ± 0.15 b | 0.07 ± 0.04 | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.07 ± 0.03 |
| *trans*-2-Hexenal | 3.34 ± 0.19 | 3.23 ± 0.52 | 3.78 ± 0.11 b | 4.10 ± 0.28 a | 0.37 ± 0.06 a | 0.28 ± 0.02 b | 0.11 ± 0.06 | 0.12 ± 0.04 |
| *cis*-3-Hexenol | 0.23 ± 0.01 | 0.21 ± 0.12 | 0.16 ± 0.03 b | 0.26 ± 0.05 a | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Hexanol | 0.04 ± 0.01 | 0.05 ± 0.02 | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| 6-Methyl-5-hepten-2-one | 0.75 ± 0.07 a | 0.44 ± 0.08 b | 2.28 ± 0.27 a | 1.74 ± 0.15 b | 0.19 ± 0.04 a | 0.10 ± 0.02 b | 0.12 ± 0.03 | 0.09 ± 0.02 |
| 2-Isobutylthiazole | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Linalool | n.d. | n.d. | n.d. | n.d. | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| 2-Phenylethanol | 0.15 ± 0.04 | 0.15 ± 0.03 | 0.14 ± 0.05 | 0.22 ± 0.04 | n.d. | n.d. | n.d. | n.d. |
| Methyl salicylate | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.16 ± 0.07 | 0.09 ± 0.04 | 0.07 ± 0.01 | 0.07 ± 0.04 |
| Geranyl acetone | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.15 ± 0.01 a | 0.07 ± 0.01 b | 0.05 ± 0.02 | 0.04 ± 0.01 | 0.07 ± 0.01 | 0.05 ± 0.01 |
| $\beta$-Ionone | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.02 ± 0.02 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.001 |
| Total | 6.91 a | 5.81 b | 8.98 | 8.34 | 0.94 a | 0.65 b | 0.47 | 0.46 |

Values followed by the different letter, in the same row and ripening stage, were significantly different ($P < 0.05$), Tukey's multiple-range test

*n.d.* not detected

because VR tomatoes showed higher firmness and hardness and reached better scores in odour and aroma at the end of the storage period. In general, sensory evaluations carried out with a tomato trained panel were in agreement with instrumental measurements, with SPME data showing positive correlations with odour results while HD and SDE data positively correlated with aroma scores. Our results support the idea that genetic improvement for disease resistance in tomato could be achieved without reducing sensory quality and aroma complexity of the fruits.

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# Comparative Post-harvest Behaviour of Traditional and Virus-Resistant *Muchamiel* Tomatoes

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**Running title:** Post-harvest of *Muchamiel* Tomatoes

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## ABSTRACT

### BACKGROUND

Nowadays, organoleptic quality is the primary objective for almost all tomato breeding programs. In this study, postharvest behaviour of a breeding line with genetic resistance to important viruses (ToMV, TSWV, and TYLCV) has been compared with the original traditional landrace (*Muchamiel*). The breeding line has been obtained by backcrossing, introgressing three resistance genes but trying to keep the quality characteristics of the traditional variety. Tomatoes were picked at random and stored at 10 ºC during 13 days. Quality analyses were made in both tomato samples: weight loss, colour, respiration rate, ethylene production, maturity index, instrumental hardness and sensory evaluation with trained panel.

### RESULTS

Fruits of the breeding line were characterized by higher hardness even with a higher maturity index. Results of sensory tests were in agreement with instrumental measurements. Organoleptic quality of *Muchamiel* virus-resistant tomatoes was at least as high as that of traditional tomatoes, reaching the best scores in odour and aroma at the 13$^{th}$ storage day.

### CONCLUSION

Although a long time has been required to develop the breeding line, results indicate that organoleptic fruit quality has been recovered through backcrossing, confirming the success of the breeding program.

**KEY WORDS:** Aroma; genetic improvement; *Solanum lycopersicum*; shelf life; texture; traditional cultivars.

## INTRODUCTION

Until few years ago, tomato breeders concentrated mainly in improving yield, fruit size, fruit appearance (attractive red colour and lack of defects), disease resistance and, more recently, fruit firmness and shelf life (to allow long-distance trading). Sometimes, if a consumer closes its eyes while eating a "modern greenhouse" tomato, it could taste as any other juicy and fresh fruit. However and according to a market survey based on consumers' preference, the organoleptic quality of a food product has been ranked higher than its nutritional value, price or safety.[1]

After its arrival to Spain, tomato began a process of diversification and adaptation to the different agro-climatic conditions of different Spanish regions. During this period, special attention was paid to organoleptic quality, and a great array of traditional tomato cultivars were originated.[2] Several traditional cultivars or landraces still survive in the orchards of Southern and Eastern Spain. Frequently ignored outside their production area, all of them are highly esteemed by local people due to their excellent organoleptic quality and are much more expensive than hybrid varieties. These landraces are highly susceptible to several viruses, such as the ToMV, TSWV and TYLCV, the incidence of which makes their cultivation non-viable.[3] As a consequence of a breeding program for the introduction of genetic resistance (genes *Tm-2*$^a$, *Sw-5*, and *Ty-1*) into traditional tomato cultivars, we have developed several breeding lines corresponding to the *Muchamiel* or *De la Pera* types (Valencian Community, Eastern Spain). The effectiveness of the introgressed resistance genes has been evaluated in several trials. The breeding lines have shown high levels of resistance to ToMV, TSWV and TYLCV in field experiments performed under different environments (open field, mesh-cover greenhouse and greenhouse), using both natural and artificial virus inoculation.[4,5]

Organoleptic quality involves taste and aroma (flavor), colour and texture of fruit. Many studies on tomato described the chemical composition of the fruit.[6]

Flavor mainly depends on sugar and acid contents but also on the sugar/acid ratio and contents of volatile compounds.

Breeding for organoleptic quality has been severely restricted by the lack of efficient criteria and by the polygenic nature of the trait. One of the present preoccupations of tomato breeders is to associate high fruit firmness, long shelf life, good flavor, and many disease resistance genes in the same variety.

In previous studies, it was demonstrated that the traditional types of Spanish tomatoes (*Muchamiel* and *De la Pera*) presented higher intensities of tomato odour and aroma according to a trained panel, they were more accepted by a consumer panel than hybrid types (*Odissea*).[7,8] Besides, the traditional tomatoes showed significantly higher contents of volatile compounds such as 3-methylbutanal, hexanal, *cis*-3-hexenal, 1-hexanol and 2-isobutylthiazole.

The global aim of this study was to demonstrate that genetic improvement for disease resistance, if organoleptic quality is considered during the breeding program, does not reduce significantly the quality of *Muchamiel* tomatoes. With this general aim in mind, the specific objective was to compare quality parameters, ethylene and respiration rates, instrumental texture, and sensory profiles of traditional and virus-resistant (VR) *Muchamiel* tomatoes during cold storage (10 ºC, 90 % relative humidity for 13 days). Results from this study should help breeders, farmers and everybody involved in farming and commercializing tomatoes to understand that genetic improvement for disease resistance could be achieved without reducing organoleptic quality. Tomato breeders should provide consumers with products of high organoleptic quality.

## MATERIALS AND METHODS

### *Plant Material and Growing Conditions*

Plants of a traditional *Muchamiel* landrace and a *Muchamiel* breeding line with genetic resistance to ToMV, TSWV and TYLCV were grown in a greenhouse in an organic farm in Lorca, Murcia (Eastern Spain), over a spring-summer growing cycle. Tomatoes were harvested on June 3, 2008. Fruits of *Muchamiel* cultivars are large in size (>200 g), flattened and strongly ribbed. Forty-day-old seedlings were transplanted at the beginning of March. Plants were allowed to grow vertically with a single stem. Pollination was improved using bumblebees (*Bombus terrestris*). Standard irrigation for tomato crop was used and only organic matter was applied before transplanting to maintain the soil fertility.

### *Sampling and Samples Processing*

Tomatoes were picked at the mature-green stage of ripening, selected according to the USDA standard tomato colour classification chart.[9] One hundred and forty units of each type of fruit (traditional and VR) at the same ripening stage were harvested. Tomatoes were picked at random from different plants of each plot. Fruits were randomly separated into 24 batches per tomato type (5 fruits per batch): 5 batches per sampling day during four samplings (0, 6, 9, and 13 days), plus 4 batches for shelf life studies. At each sampling day, 25 fruits (5 batches) were used to analyze the quality parameters. Samples were stored at 10 ºC and 90 % RH during 13 days. Once in the laboratory, each batch was weighted and the individual diameter of each fruit was measured.

***Quality Parameters***

First, instrumental colour of whole tomatoes was measured in triplicate in all tomatoes (25 replicates per sampling) using a Minolta CR-300 colorimeter (Minolta Camera Co Ltd, Osaka, Japan).

For quality parameters, 10 fruits per tomato type (traditional and VR) were picked randomly from each sampling population and were individually analyzed (10 replicates). Immediately after the fruits had been juiced using a domestic juice extractor, the total soluble solids content (TSS) was determined with an Atago PR-100 digital refractometre (Atago Co Ltd, Tokyo, Japan); the results were expressed as ºBrix. Titratable acidity (TA) was measured by titration with 0.1 N NaOH using phenolphthalein until pH 8.1; data were presented as percentage of citric acid.



***Ethylene and Respiration Rates***

Ten whole tomatoes per tomato type were randomly chosen and used for determining the ethylene and respiration rates. Ethylene production was measured using a Hewlett-PackardTM model 5890A GC-FID (Wilmington, DE), while the respiration rate was quantified using a ShimadzuTM 14B GCTDC. The specific conditions of these analytical protocols could be found in Valero et al..[10] Results were reported as the mean±standard error using the following units: nmol $g^{-1}$ $h^{-1}$ for ethylene and μmol $kg^{-1}$ $h^{-1}$ for respiration rate. These analyses were run in 10 replicates (1 fruit per replicate).

***Instrumental Texture***

The instrumental texture of each single tomato was studied using a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, U.K.) of 25 kg load cell capacity. Ten fruits per cultivar were analyzed at each sampling day (2 replicates per fruit).

Two different tests were used with both cultivars: Magness–Taylor test (MTT) and compression test (CT):

- The MTT assay is an empirical hardness indicator, and measurements were recorded using a 5-mm-wide cylindrical probe (P2). This test involves both compression (probe centre) and cutting (probe edge) efforts. The penetration rate was 0.3 mm $s^{-1}$ and lasted for 5 s after contacting the surface of tomatoes. Results were expressed in N.[11]
- The CT is an instrumental texture parameter also related to the hardness of the product. The samples were compressed 3 % of their original height using a 10-cm-wide cylindrical probe (P100) and compressing at a speed of 0.3 mm $s^{-1}$. Results were expressed in N $mm^{-1}$.

***Sensory Evaluation with Trained Panel***

Sensory evaluation with trained panel was used to discriminate the intensities of "fresh tomato" odour (perception of volatile compounds sniffed through the nose), "fresh tomato" aroma (perception of volatile compounds during chewing of samples) and other organoleptic properties such as: colour, hardness, sweetness, acidity, etc. At each sampling day, the trained panel studied the two tomato types under study: traditional and VR.

A panel of 10 panellists, ages 20 to 50 years (6 female and 4 male, all members of the University Miguel Hernandez), with sensory evaluation experience, was trained in descriptive evaluation of tomato.

The panel was selected and trained following the ISO standard 8586-1.[12] Details on panel selection, training and validation could be found in Alonso et al.[8]

Measurements were performed in individual booths with controlled illumination and temperature.[12,13]

The individual products were scored for the intensity of different organoleptic parameters (colour, odour, sweetness, acidity, aroma, hardness and juiciness) on a scale of 0 to 10, where:

- 0 = extremely low intensity.
- 5 = regular intensity in fresh tomatoes.
- 10 = extremely high intensity.

***Shelf Life Studies***

Shelf life studies were carried out with the aim of studying the behaviour of tomatoes at consumers' homes. Days 6 and 13 of storage, two batches were removed from the cooling chamber and kept in the laboratory at room temperature (20-23 ºC) during 2 days. After this time, all previously mentioned analyses were carried out, at least in 5 replications per analysis.

***Statistical Analyses***

Data from tomatoes analyses were examined by analysis of variance (ANOVA) using STATGRAPHICS Plus 5.0 software (Manugistics, Inc., Rockville, MD). Wherever *F* values were significant, Tukey's Multiple Range Test was used to separate the mean effects. Significance was defined at $p \leq 0.05$. Graphics were done using Sigma Plot 9.0 (SPSS Science, Chicago, IL, USA).

## RESULTS AND DISCUSSION

### *Fruit physico-chemical properties*

As can be seen in **Table 1**, the main quality parameters at the beginning of the study (day 0) were as identical as possible. Fruits were picked and later selected in the laboratory according to their colour; even there were some statistically significant differences in the values of coordinates *L** and *b** in fruits, they were lower than 2 units and therefore had no biological importance. However, there were not significant differences in the most important coordinate *a**, which represents the ripening process going from green to red colours. Virus-resistant *Muchamiel* tomatoes presented slightly higher values of total soluble solids (TSS) and lower of titratable acidity (TA), which led to slightly higher values of the maturity index; but again these differences were minimal and had not significant biological meaning.

The main quality parameters of both types of tomatoes under study were quite similar during storage at 10 ºC and 90 % RH; even though sometimes there were minor differences, they showed no clear trend. The main difference was found in the maturity index, which was always higher in VR tomatoes because the titratable acidity was always lower since the first day of storage.

The genetic improvement had no significant effect on weight loss until the last day of storage, in which VR tomatoes showed only a loss of 6.76±0.75 % compared to 8.04±0.40 % in traditional tomatoes.

Fruit colour did not show significant differences between tomato types during their cold storage. Lightness coordinate, *L**, remained constant and ranged between 49.8 and 53.9. The coordinate *a** (green-red) significantly increased during the storage period (from around -1 up to 20) but showed not clear differences among traditional and VR *Muchamiel* tomatoes. Finally, the coordinate *b** (blue-yellow) was almost constant and ranged between 30.8 and 35.2.

***Ethylene production and respiration rate***

The levels of ethylene production at harvest decreased when fruit were stored at 10 ºC, reaching the lowest values after 1 day of cold storage and increasing slightly until day 13. Initial values (day 0) were 510±10 and 505±10 nmol $kg^{-1}$ $h^{-1}$ for traditional and VR tomatoes, respectively; these values decreased to 9.8±1.0 and 11.7±0.7 nmol $kg^{-1}$ $h^{-1}$ at day 1, and increased up to 48.9±7.4 and 32.0±1.9 nmol $kg^{-1}$ $h^{-1}$ at the end of the storage period (13 days) (**Figure 1**). A similar pattern (initial decrease and later slight increase) was previously described by Guillén et al. in other Spanish tomato cultivars (Cherry, Daniela, Patrona and Raf).[15] Ethylene production of climacteric fruits is not inhibited with low temperature storage, and the associated ripening process remained.[16] At the end of the storage period, there were statistically significant differences in ethylene production for traditional and VR tomatoes; however, the differences were not high enough to justify significant changes in colour, maturity index, softening, etc.

A similar behaviour was found for respiration rate, with significant decreases happening after cold storage and constant rates thereafter. Respiration rate in recently harvested tomatoes were 912±12 and 950±15 µmol $kg^{-1}$ $h^{-1}$ for traditional and VR tomatoes, respectively; ethylene production at the same time of harvest was 510±10 and 505±10 nmol $kg^{-1}$ $h^{-1}$ for traditional and VR tomatoes respectively. These values decreased to 139±12 and 127±4 nmol $kg^{-1}$ $h^{-1}$ at day 1, and increased up to 146±13 nmol $kg^{-1}$ $h^{-1}$ and 131±2 nmol $kg^{-1}$ $h^{-1}$ at the end of the storage period (13 days) (**Figure 1**). No statistically significant differences were found between samples due to genetic improvement of tomato plants.

***Fruit texture***

Two instrumental texture parameters were considered in this study: Magness-Taylor test (MTT) and Compression test (CT). The first one, MTT, is related with peel resistance and firmness, because compression and cutting efforts (centre and edge of the probe, respectively) are involved. On the other hand, the CT is mainly related with fruit hardness or firmness because the whole product is compressed by a probe bigger than the fruit. Tomato ripening after harvesting is often associated with fast and important quality losses, including significant softening of fruits. The more intense the ripening process, the shorter the shelf life.

Typical softening patterns (general decreases of both MTT and CT) were found in both types of tomatoes during cold storage, 10 ºC (**Figure 2**). Breeding for virus resistance of the *Muchamiel* landrace has resulted in fruits with high hardness and firmness, although during the breeding program, no intentional selection for hardness and firmness was carried out. However, these better characteristics could be quite useful during post-harvest manipulation of fruits. MTT values for traditional and VR tomatoes were 5.36±0.23 N and 6.13±0.29 N at day 0 and constantly decreased down to 3.98±0.17 N (decrease of 25.7% for traditional fruits) and 5.20±0.22 N (decrease of 15.2% for VR fruits) at day 13 of cold storage. CT values for traditional and VR tomatoes were 11.58±1.20 N and 14.21±1.01 N at day 0 and constantly decreased down to 7.22±0.41 N for traditional fruits and 8.95±0.44 N for VR fruits at the end of the storage period.

The higher values of MTT and CT in VR *Muchamiel* tomatoes could be related to the lower respiration and ethylene production rates in this type of tomatoes, and without any doubt implies a significant improvement in their quality, which will improve the post-harvest behaviour of these fruits.

***Sensory evaluation***

Flavor quality of fruits and vegetables is influenced by genetic, pre-harvest, harvesting and post-harvest factors. The post-harvest life based on flavor and nutritional quality is usually shorter than that based on appearance and textural quality. It is essential that good flavor quality will be taking into account by selecting the best-tasting genotypes.[17] Thus, organoleptic quality is an essential quality factor in tomatoes and several studies have been performed about this subject.[7,17-19]

At the first day of storage, VR tomatoes showed higher sweetness than traditional fruits (6.6 compared to 4.7), fact that was corroborated by the instrumental measurements (3.95 ºBrix compared to 3.77 ºBrix). During storage, no clear trend of the sensory sweetness was observed and scores (even though total soluble solids and the maturity index increased during the storage time) ranged from 4.5 to 6.6, with mean values of 5.3 and 5.6 for traditional and VR tomatoes respectively **(Figure 3).** These sweetness values showed the moderate sweet taste that is characteristic of the *Muchamiel* type; it is necessary to recall that 5 is the regular intensity of fresh tomatoes.

Instrumental acidity was always higher in traditional tomatoes than in VR fruits. However, the trained panel was only able to corroborate this trend, at day 0 (6.3 compared to 4.3). After cold storage started, both tomato samples were scored equally in acidity (no statistically significant differences were found), and scores ranged between 4.7 and 6.2. Acidity in fruits is determined by the concentrations of the predominant organic acids and some amino acids as aspartic and glutamic, but minerals such as calcium, phosphorus and potassium, combine with the organic acids, have influence in acidity perception.[17]

Even though values of the green-yellow coordinate, *a**, were always higher in VR tomatoes, the trained panel was not able to find significant differences in colour intensity between traditional and VR *Muchamiel* tomatoes. The experimental

disagreement among sensory and instrumental results showed one more time than instrumental colour is more reliable than sensory colour.

The juiciness was not influenced at all by the genetic improvement of *Muchamiel* tomatoes; scores for this parameter ranged between 5.3 and 6.6, with mean values being 6.1 and 5.9 for traditional and VR *Muchamiel* tomatoes.

Considering the fact that the general aim of this study was to demonstrate that genetic improvement will not reduce significantly the quality (flavor and texture) of *Muchamiel* tomatoes during cold storage, aroma, odour and hardness could be considered as the most relevant organoleptic attributes. **Figure 3** shows that hardness scores were always higher in VR tomatoes than in the original landrace (6.3 compared to 5.1). This higher hardness will be an advantage during post-harvest operations, including washing, calibration, cold storage, distribution, etc. The odour (perception of volatile compounds while smelling tomatoes) of traditional *Muchamiel* tomatoes significantly decreased during the cold storage at 10 ºC and 90 % RH, from an initial value of 6.7 at day 0 down to 5.1 at day 13. At days 0 and 6, the traditional tomatoes presented higher odour intensities than VR fruits. However, this clear trend completely changed at the last days of storage; at days 9 and 13, VR tomatoes showed higher odour intensities than traditional fruits. A similar behaviour was observed for aroma (perception of volatile compounds while chewing the tomatoes), with the aroma intensity decreasing with the time of cold storage in traditional tomatoes (from 6.3 to 4.9). At the first two sampling periods, traditional tomatoes had higher aroma intensities, while at the last two samplings VR tomatoes presented more intense aroma. These experimental results indicate that the process of genetic improvement for virus resistance of the *Muchamiel* landrace has resulted in tomatoes with more stable and resistant odour and aroma.

***Shelf life***

The storage (48 h) of tomatoes at room temperature promoted the ripening of fruits, as expected. The behaviour of both tomato types under study (traditional and VR) was quite similar regarding the main quality parameters (for instance, values at day 13+2 were as follows (for traditional and VR tomatoes, respectively): total soluble solids (4.36±0.13 and ± 4.10±0.04 ºBrix), titratable acidity (0.22±0.01 and 0.22±0.01 %), maturity index (20.38±1.04 and 19.00±0.41), and fruit colour (*L** 45.80±0.86 and 46.10±0.69; *a** 23.62±0.98 and 23.6±0.70; *b** 25.91±1.04 and 28.14±0.91) but the changes on respiration and ethylene rates and hardness were more significant in traditional than in VR tomatoes, especially day 13+2. At this day (13+2), ethylene production rates were 123±5 and 84.4±3.6 nmol $kg^{-1}$ $h^{-1}$ for traditional and VR *Muchamiel* tomatoes, respectively; while the respiration rates were 334±10 and 301±9 µmol $kg^{-1}$ $h^{-1}$ for traditional and VR tomatoes, respectively.

Storage at room temperature, decrease the values of both firmness and compression force but, in general, values were significantly higher in VR *Muchamiel* tomatoes compared to traditional fruits (**Figure 4**).

## CONCLUSIONS

A long time has been required to introgress three resistance genes into a *Muchamiel* traditional landrace. However, the comparison of quality parameters, ethylene and respiration rates, instrumental texture, and sensory profiles of traditional and VR tomatoes during cold storage indicate that organoleptic fruit quality has been recovered through the backcrossing program. Even more, fruits of the breeding line demonstrated a better postharvest behaviour because VR tomatoes showed higher firmness and hardness and reached better scores in odour and aroma at the end of the storage period. In general, sensory evaluations carried out with a tomato trained panel were in agreement with instrumental measurements, confirming the utility of the panel of experts for breeding purposes. Our results support the idea that genetic improvement for disease resistance in tomato could be achieved without reducing organoleptic quality of the fruits.



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**Table 1**. Quality parameters on fruits during storage at 10 ºC and 90 % RH.

| Parameter | Tomato Type | Storage Time (days) | | | |
|---|---|---|---|---|---|
| | | 0 | 6 | 9 | 13 |
| Weight loss (%) | Traditional | 0 | 4.42 a C | 5.44 a B | 8.04 a A |
| | VR | 0 | 4.40 a B | 5.93 a A | 6.76 b A |
| Soluble Solids (º Brix) | Traditional | 3.77 b B† | 4.43 a A | 4.28 a A | 4.50 a A |
| | VR | 3.95 a B | 4.06 b B | 4.22 a AB | 4.42 a A |
| Titratable Acidity (%) | Traditional | 0.48 a A | 0.53 a A | 0.32 a B | 0.24 a C |
| | VR | 0.44 a A | 0.41 b A | 0.24 b B | 0.19 b B |
| Maturity Index | Traditional | 7.91 b D | 8.39 b C | 13.3 b B | 18.0 b A |
| | VR | 8.94 a D | 9.92 a C | 17.8 a B | 24.2 a A |
| *L** (fruit) | Traditional | 53.92 a A | 50.48 a A | 52.48 a A | 51.52 a A |
| | VR | 52.11 b A | 50.60 a A | 49.80 b A | 51.65 a A |
| *a** (fruit) | Traditional | -1.46 a C | 16.59 a A | 10.50 a B | 16.61 b A |
| | VR | -0.25 a D | 13.97 b B | 9.04 a C | 19.47 a A |
| *b** (fruit) | Traditional | 31.93 b A | 30.84 a A | 32.91 b A | 31.65 b A |
| | VR | 33.65 a A | 32.15 a A | 34.40 a A | 35.22 a A |

†*Values followed by the same "small" letter, in the same column and within the same variation source (effect of breeding), were not significant different ($p < 0.05$), Tukey's multiple-range test. Values followed by the same "capital" letter, in the same row (effect of storage time), were not significant different ($p < 0.05$).*

**Figure 1.** Respiration rate and ethylene production of tomato cultivars during storage at 10 ºC and 90 % RH.

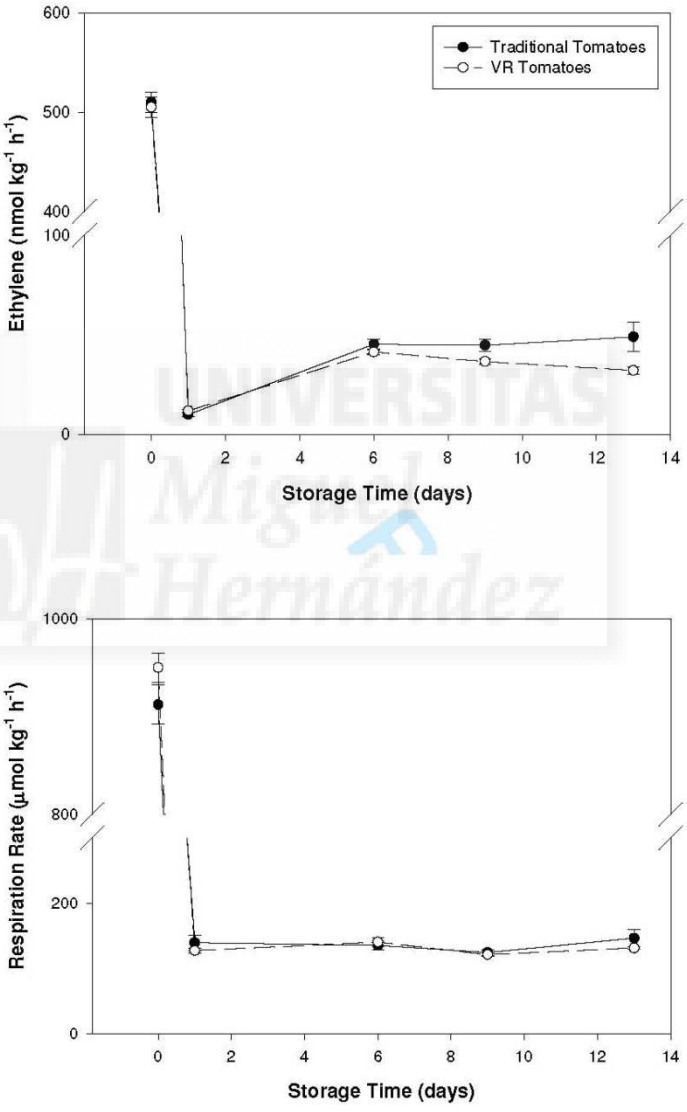


**Figure 2.** Fruit firmness (MTT) and compression force (CT) during storage at 10 ºC and 90 % RH.

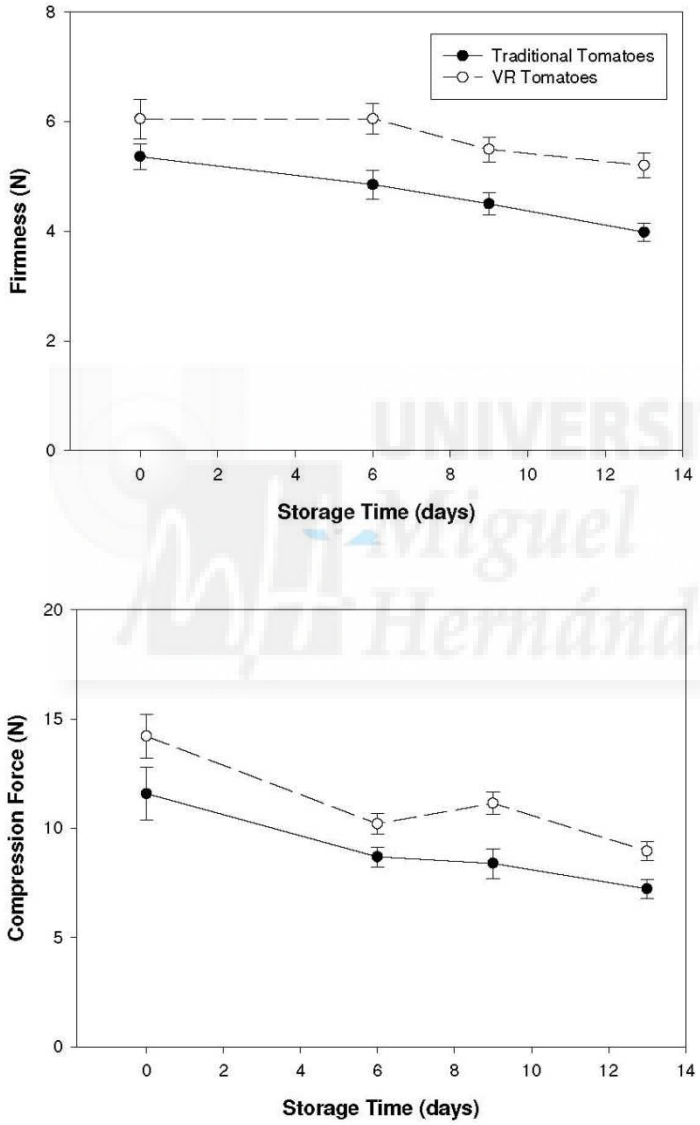


**Figure 3.** Sensory profiles of *Muchamiel* tomatoes during storage (0, 6, 9 and 13 days) at 10 ºC and 90 % RH.

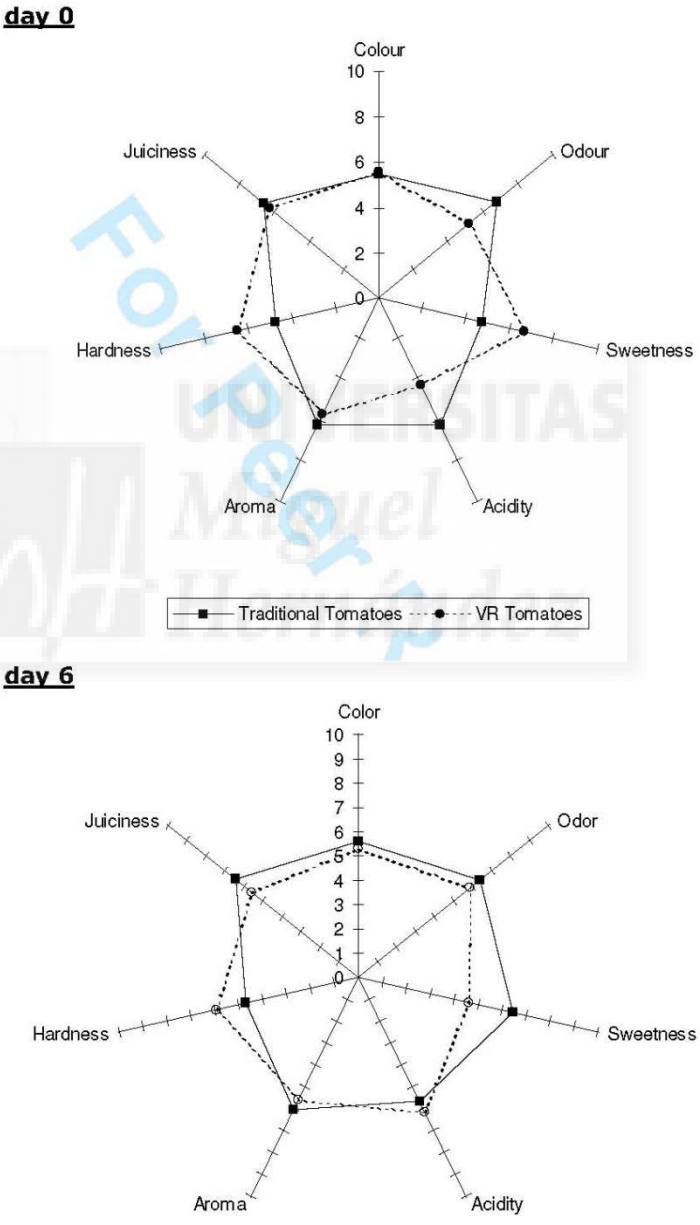


**Figure 3.** (continuation).

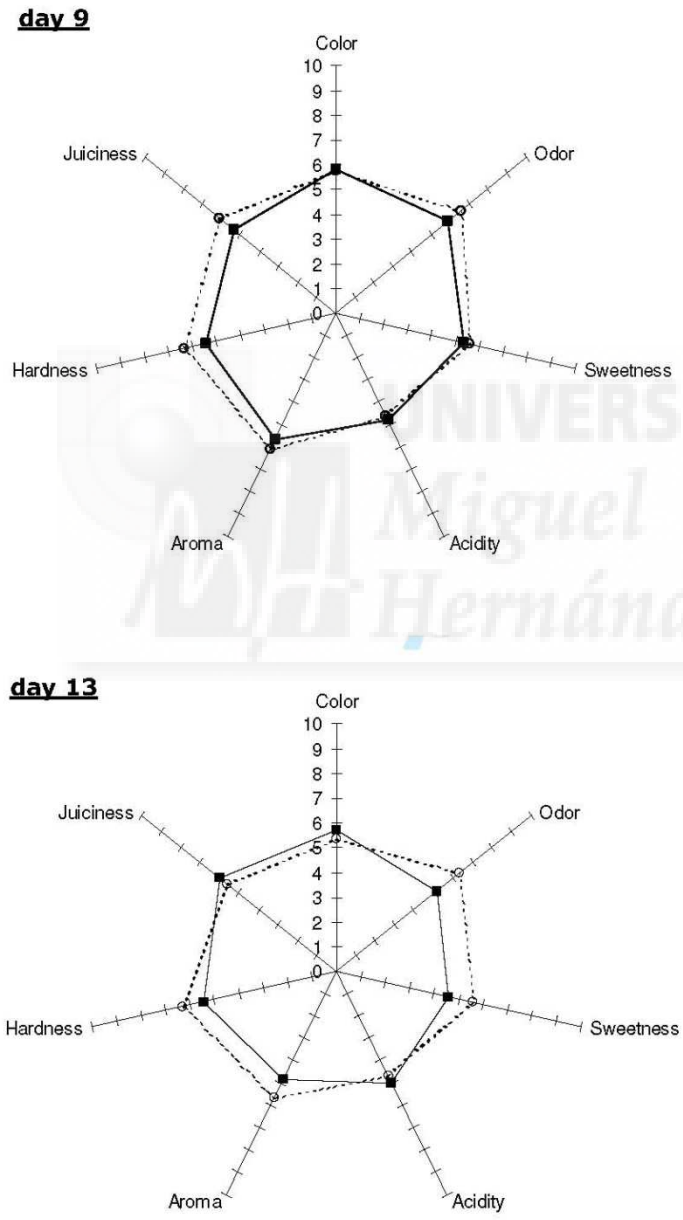
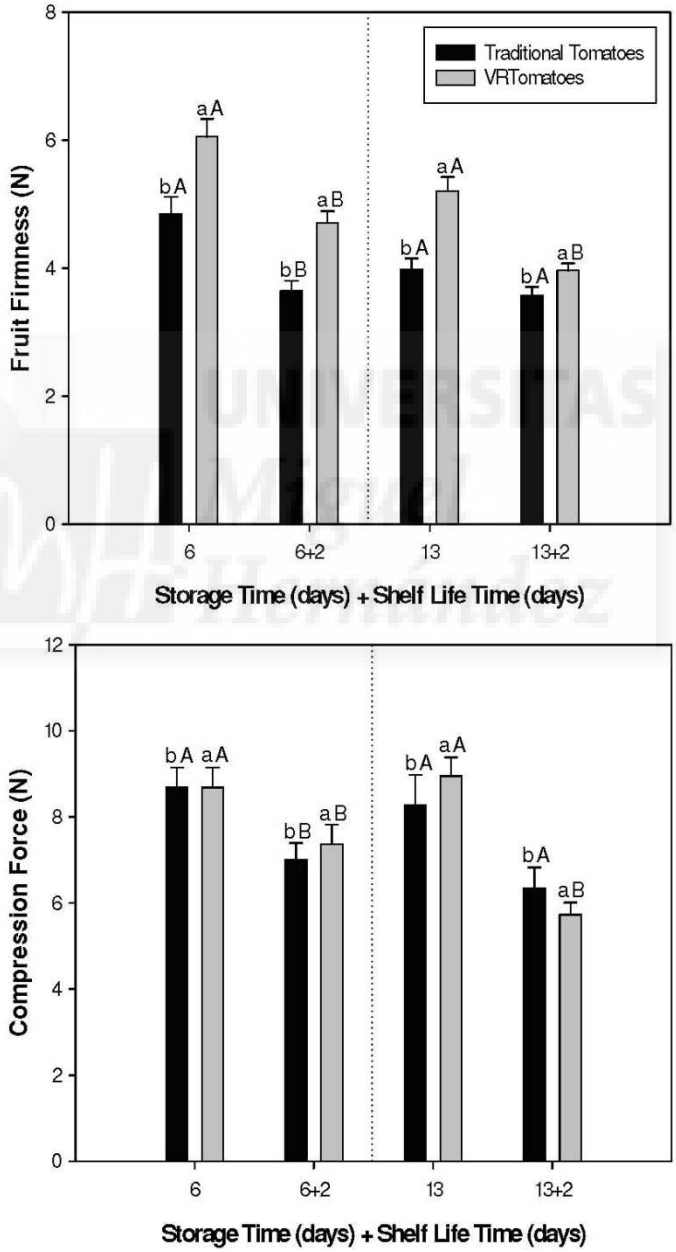


**Figure 4.** Fruit firmness (MTT) and compression force (CT) during shelf life. The statistical analysis was independently carried out for each storage time. *Values followed by the same "small" letter, in any storage and shelf life times (6, 6+2, 13, 13+2), were not significant different ($p < 0.05$. Values followed by the same "capital" letter, in the same storage time (6 or 13), were not significant different ($p < 0.05$).*



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Dear Professor Carbonell-Barrachina,

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## 3.- RESUMEN DE RESULTADOS, DISCUSIÓN Y CONCLUSIONES

El Programa de Mejora de variedades tradicionales iniciado en 1998 está en sus últimas etapas, en las cuales se deben seleccionar las líneas con mejores características agronómicas y de calidad fisico-química y sensorial. Se dispone de líneas de los genotipos “De la Pera” y “Muchamiel” resistentes a ToMV, TSWV y TYLCV que han recuperado gran parte de las características morfológicas y organolépticas de las variedades originales. Estos materiales están siendo cultivados con éxito por agricultores de distintas zonas del sureste de España, fundamentalmente en las provincias de Alicante y Murcia, que están vendiendo sus producciones a buen precio. Estas colaboraciones posibilitarán el desarrollo final de distintas variedades resistentes y adaptadas a diferentes condiciones de cultivo (resultados recogidos en García-Martínez *et al*., (2008), trabajo publicado en ***Agrícola Vergel***).

Analizar la contribución del contenido aromático en el *flavor* del tomate requiere equipos costosos y un laborioso trabajo de los jueces sensoriales entrenados, de modo que sólo resultará una herramienta práctica dentro de un programa de mejora si el número de muestras a analizar es escaso. Además, para seleccionar genotipos de forma individual se necesitarían métodos de análisis que precisen de una reducida cantidad de muestra de tomate. En Ruiz *et al*. (2005), trabajo publicado en el ***Journal of the Science of Food and Agriculture***, utilizando el método de extracción purga-trampa y un reducido número de frutos por cultivar en un estado de madurez lo más uniforme posible, pudimos detectar diferencias significativas entre accesiones, muy estrechamente relacionadas entre sí, de los tipos “Muchamiel” y “De la Pera“ para 5 compuestos volátiles seleccionados. A pesar de que se requiera seguir investigando, resulta interesante que el cultivar más apreciado en las pruebas organolépticas, el “Muchamiel-4”, fue el que alcanzó también el contenido más elevado en hexanal y *cis*-3-hexenal.

Los mejoradores de tomate consideran el *flavor* como uno de los puntos clave de la calidad del fruto, puesto que los consumidores se preocupan cada

vez más de los aspectos sensoriales. Dado que el aroma del tomate es muy complejo, reducir el número de compuestos a estudiar a solo unos pocos con un protagonismo principal, incrementaría la utilidad de las determinaciones de volátiles en la mejora. Mediante análisis discriminante hemos sido capaces de generar un modelo matemático para distinguir con éxito el tipo de tomate al que pertenece un fruto dado, usando únicamente las concentraciones de 6 volátiles: 3-metilbutanal, 1-penten-3-ona, hexanal, *trans*-2-hexenal, 1-hexanol y 2-isobutiltiazol. Así, en base a las concentraciones de estos pocos volátiles, podíamos clasificar un fruto como perteneciente al tipo "Muchamiel" o al tipo "De la Pera". Los frutos de los tipos tradicionales presentaron las mejores puntuaciones en la valoración organoléptica, así como el contenido más elevado para la mayoría de los compuestos volátiles estudiados. Estos resultados, de interés para la mejora, se incluyen en el trabajo Alonso *et al*. (2009a), publicado en la revista ***Food Science and Technology International*****.**

Se necesita mucho tiempo para introgresar tres genes de resistencia en una línea de tomate tradicional. Además, en ocasiones la introducción de la resistencia ocasiona una disminución de algunos caracteres de calidad, respecto a la variedad original. Comparando una variedad tradicional de tipo "Muchamiel" con su línea de mejora con los tres genes de resistencia introgresados, encontramos que, en general, las determinaciones sensoriales llevadas a cabo con el panel entrenado concordaron con las medidas instrumentales realizadas. Los datos de la técnica extractiva de extracción y destilación simultánea (SDE) mostraron correlaciones positivas con los resultados obtenidos para el olor. Así mismo, los datos obtenidos empleando los métodos de hidrodestilación (HD) y de microextracción en la fase sólida (SPME) se correlacionaron positivamente con las puntuaciones de aroma. En general, el hexanal fue el principal compuesto en los tomates tradicionales mientras que el 6-metil-5-hepten-2-ona lo fue en los mejorados y ambos tienen descriptores similares. Aunque las concentraciones de volátiles totales fueron mayores en el SDE, los resultados de la técnica de HD mostraron una mejor correlación con los datos sensoriales (Alonso *et al*., (2009b), publicado en la revista ***European Food Research and Technology***).

La comparación de parámetros de calidad, como el contenido en sólidos solubles y la acidez valorable, la producción de etileno, la tasa de respiración, la textura y los perfiles sensoriales de los tomates de una variedad tradicional y de una línea de mejora con resistencias genéticas, durante el almacenamiento en refrigeración, indicaron que la calidad organoléptica de los frutos había sido recuperada durante el programa de retrocruzamiento. Los frutos de la línea de mejora mostraron incluso mayor firmeza y dureza, y alcanzaron mejor puntuación en cuanto a olor y aroma al final del período de almacenaje. De nuevo las evaluaciones sensoriales llevadas a cabo estuvieron de acuerdo con las medidas instrumentales, confirmando la utilidad del panel de cata para el programa de mejora. Nuestros resultados, aceptados para publicación en la revista ***Journal of Science Food & Agriculture*** (Alonso *et al.,* en prensa), apoyan la idea de que la mejora genética para introducir resistencias en tomate puede lograrse sin reducir la calidad sensorial de los frutos.

En las últimas etapas del Programa de Mejora de variedades tradicionales donde nos encontramos se deben seleccionar las líneas con mejores características agronómicas y de calidad. La determinación de la calidad es muy compleja, debido a la cantidad y naturaleza de los factores implicados, y además, las técnicas empleadas deben requerir una cantidad de muestra lo más reducida posible. Las diversas técnicas de análisis de la calidad puestas a punto y aplicadas en los materiales procedentes de nuestro programa de mejora han demostrado su utilidad, por lo que podrán ser usadas en las últimas etapas de selección.

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