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PAPER

FUNCTIONAL AND RHEOLOGICAL CHARACTERISTICS OF FRESH EGG PASTA

CARATTERISTICHE FUNZIONALI E REOLOGICHE DI PASTA FRESCA ALL'UOVO

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ABSTRACT

The composition, colour, cooking behaviour and rheological properties of different samples of industrial packaged and retail-manufactured fresh egg pasta were evaluated. All of the parameters showed high variability, due to differences in product formulation and technology. Retail-manufactured pastas were clearly distinguished from packaged samples due to the absence of pasteurisation. Pasteurisation caused raw pasta to become tougher and harder, while its break strain and matter

RIASSUNTO

La pasta fresca all'uovo risulta poco studiata nella letteratura scientifica. Lo scopo del lavoro è stato quello di valutare la composizione, il colore, il comportamento in cottura e le proprietà reologiche di differenti campioni di pasta fresca all'uovo preconfezionata e artigianale. Tutti i parametri considerati hanno mostrato un'elevata variabilità, imputabile soprattutto alle differenti formulazioni e tecnologie adottate. In particolare, l'assenza della pastorizzazione nei campioni artigianali li distin-

- Key words: cooking behaviour, egg, fresh pasta, furosine, pasteurisation, rheology -

loss in cooking water decreased. Principal Component Analysis separated the pasta samples into three groups, according to the severity of the thermal treatment applied. It was observed that cooking caused an overall reduction in the variability of the rheological characteristics, making retail-manufactured and packaged pastas more similar to one another.

gue nettamente da quelli preconfezionati. Infatti, a seguito del trattamento termico, la pasta risulta più tenace e dura, mentre si osserva una diminuzione della deformazione alla rottura e del residuo solido rilasciato in cottura. L'Analisi delle Componenti Principali ha permesso di distribuire i campioni di pasta in tre gruppi, in relazione all'intensità del trattamento termico subito. Infine è stato osservato come la cottura della pasta provochi una generale riduzione della variabilità delle caratteristiche reologiche, rendendo più simili le paste artigianali e quelle preconfezionate.

INTRODUCTION

Fresh pasta is a very common and very much appreciated product, especially in Italy, where it has a growing market with good development possibilities. At the European level, there are no regulations that apply specifically to pasta. In Italy, this sector is regulated by DPR n. 187 of 9 February 2001 (DPR, 2001). For fresh egg pasta, in particular, the law states that it must be produced using semolina and/or wheat flour and at least four whole, shelled hen's eggs, for a total weight of no less than two hundred grams of egg for every kilogram of flour mix. The eggs may be replaced by an equivalent quantity of liquid egg product, produced exclusively with whole hen's eggs, and satisfying the requirements prescribed by EEC Directive 437/89 (EEC, 1989).

In addition to the indexes provided for other types of pasta (ash, protein and acidity), fresh egg pasta must have an ether extract no lower than 2.80 g/100 g d.m. and a sterol content of at least 0.145 g/100 g d.m.

Within the category "fresh egg pasta", the law provides for two types of products:

- loose pasta: this is sold loose; from production to sale, it must be kept at a temperature no higher than 4°C;
- packaged pasta: this is sold prepacked; it must have a moisture content no lower than 24% and a water activity (a_w) between 0.92 and 0.97; this pasta must have undergone a thermal treatment at least equivalent to pasteurisation and must be kept, from production to sale, at a temperature no higher than 4°C.

Since the legislation does not cover the processes permitted or required for the pasteurisation of packaged pasta, the various manufacturers use different technologies. The main difference lies in the number of thermal treatments applied: pasteurisation may be carried out on the loose product only, using an injected steam belt pasteuriser or, after packaging, the pasta may be treated a second time in static cells. Moreover, each treatment may be carried out using different combinations of time and temperature. The intensity of the thermal treatment applied can be effectively evaluated by determining the quantity of furosine (ε-N-furoylmethyl-L-lysine). Actually, Amadori

compounds formed in the Maillard reaction can be converted by acid hydrolysis into the stable furosine. This molecule is considered a useful marker of heat damage undergone by various products (RESMINI and PELLEGRINO, 1991).

The different pasteurisation conditions applied, together with the different formulations and production technologies employed, definitely affect the characteristics of the fresh egg pastas found on the market. However, fresh egg pasta has been little investigated, and the studies that have been carried out have often focussed on fresh filled pasta (CENCIC *et al.*, 1995; DE CINDIO *et al.*, 2001; ZARDETTO *et al.*, 2003) or on the purely microbiological aspects (MAGRI *et al.*, 1999; NANNI *et al.*, 2000; ALTIERI *et al.*, 2002). However, there are no reports in the literature which correlate the effects of pasta pasteurisation with the rheological and functional characteristics of the pasta itself.

The composition of various samples of packaged and retail-manufactured fresh egg pasta on sale to the public, together with colour indexes, cooking behaviour and rheological properties, were evaluated with the aim of investigating the variability of these characteristics and evaluating any effect pasteurisation has on them.

MATERIALS AND METHODS

Packaged fresh egg pasta

Twenty different commercial brands of lasagna-style fresh egg pasta were purchased on the market. According to the labels, eight samples were made with semolina, while the others contained semolina and flour in different ratios. The egg ingredient ranged from 17 to 30%, with an average of 21.8%; three brands did not declare the amount of egg. Samples are here identified by the letter "B", followed by a progressive number representing the brand. Two samples, labelled as organic pasta, are also identified by the abbreviation "org".

Retail-manufactured fresh egg pasta

Five samples of lasagna-style fresh egg pasta were purchased from different retail pasta manufacturers. These pasta samples were sold loose and their ingredients and expiration dates were not declared; they are here identified by the letters "RM", followed by a progressive number.

During analyses, commercial and retail-manufactured pasta samples were kept in airtight polystyrene containers to avoid moisture loss.

Moisture

Moisture content of pasta was determined by a gravimetric method, according to the Italian Metodi Ufficiali di Analisi dei Cereali (DM, 1967). Results are the average of two determinations and are expressed as g/100 g.

Protein and lipid content

Total nitrogen content of pasta was evaluated according to AOAC Official Method 920.87 (AOAC, 1995). Protein content was calculated using the cereal conversion factor 5.7.

Lipid content, expressed as ether extract, was determined according to the Italian Metodi Ufficiali di Analisi dei Cereali (DM, 1967).

Protein and lipid analyses were performed in duplicate and results are expressed as g/100 g d.m.

Water activity

Water activity (a_w) was evaluated by a Water Activity Meter (series 3TE Deca-

gon Devices, Inc., Pullman, WA), previously calibrated with distilled water (a_w = 1.000) and with a standard solution of LiCl 8.57 m (a_w = 0.504). Results are the average of three replicates.

Furosine

Furosine, expressed as mg furosine/100 g of protein, was determined following the HPLC method of RESMINI and PELLEGRINO (1991), slightly modified by HIDALGO *et al.* (1995). Results represent the average of two determinations.

Thickness

Thickness of lasagna samples was measured on three different sheets of pasta using a calliper. Results are expressed in mm.

Colour

Colour determination was performed before and after pasta cooking. CIE L*a*b* indexes were determined using a reflectance colorimeter Chroma Meter CR 210 (Minolta Camera Co. Ltd, Japan), with standard illuminant C. Results represent the average of fifteen determinations carried out on three different sheets of pasta.

Cooking tests

Cooking tests were performed in standard conditions: four sheets of pasta (20x10 cm) were laid in a lasagna cooking device made of perforated stainless steel plates placed one on top of the other. The cooking device was dipped in 1,500 mL of boiling natural spring water (pasta/water ratio ca. 1/10) with no salt added. After 3 min of cooking, the cooking device was extracted from the water and the pasta sheets were left to drain for 2 min. Only the three upper sheets of cooked pasta were considered for analyses.

Weight increase

Pasta weight increase due to cooking was evaluated on the sheet of pasta set in the top position in the cooking device, weighing pasta before and after cooking. Weight increase was expressed as the percentage of the raw pasta weight and represents the average of four cooking trials.

Matter loss

Matter loss during cooking was evaluated by determining the dry matter content of the cooking water left, after restoring it to the initial volume by adding natural spring water. Dry matter was determined on 25 mL of cooking water, previously evaporated and then desiccated to constant weight in an oven at 105°C. Results, expressed as grams of matter loss/100 g pasta d.m., represent the average of four determinations, carried out on water of two different cooking tests.

Rheological tests

Tensile tests were performed on both raw and cooked pasta, using an Instron Universal Testing Machine 4301 (Instron Ltd., High Wycombe, UK) supported by Series IX Automated Material Testing software (Instron Co., 1998). Analyses were carried out at room temperature with a constant cross-head speed of 20 mm/min. Samples were shaped as "dog bone" and clamped at the ends by adjustable grips. For each sample at least six replicates were carried out. Modulus (MPa), an index of pasta hardness, was calculated on the linear part of the tensile curve as the ratio between tensile stress and relative deformation. Break strain (%) was obtained by the ratio between maximum extensibility and initial sample length. Break load (N) was also determined, as an index of pasta toughness.

Statistical analyses

One way ANOVA, Pearson correlations and Linear Discriminant Analysis (LDA) were performed using Systat 5.03 software for Windows (Systat Inc., USA). Principal Components Analysis (PCA) was carried out by Unscrambler 6.11 (Camo, ASA, Norway) applying the full cross validation.

RESULTS AND DISCUSSION

The packaged fresh egg pasta samples had rather variable declared shelf-lives, depending on the packaging techniques (aseptic and non) and on the conditions and number of thermal treatments applied during the production process. Shelf-life normally varied from 15 to 55 days for those products subjected to only one pasteurisation, while it varied from 60 to 90 days for products subjected to double pasteurisation, before and after packaging.

Preliminary tests, aimed at evaluating the stability of product characteristics during shelf-life, demonstrated no significant changes except for moisture and rheological parameters within the first four days after production (data not shown). Thus all the packaged pasta samples were analysed between one week and one month after manufacture and, always, before the expiration date. Instead retail-manufactured samples were analysed the day after production, since they are intended for short-term consumption.

Table 1 shows the values for the composition parameters and the furosine levels found in the samples of fresh egg pasta analysed, both packaged and retail-manufactured. From the results obtained, it can be noted that the samples differed considerably from one another, owing to the different ingredients used (semolina or semolina and flour in varying quantities; the presence or absence of water added to the dough), the varying amounts of egg used (declared quantity varied between 17 and 30%) and the different thermal treatments to which the samples were subjected.

The moisture content of all the packaged pasta samples was way above the legally-prescribed minimum (24 g/100 g), with a low intersample variation (CV = 5.25%). The average moisture content was very similar to that found in the loose fresh pasta, for which no legal limitations are prescribed.

The a_w values (data not reported) in the samples of packaged pasta all fell between 0.96-0.97; only sample B5, with a value of 0.98, was above the legal limit. The retail-manufactured pastas, for which no legal limit is prescribed, had an average a_w value of 0.98.

The protein content of the samples was always over the legally-prescribed minimum (12.50 g/100 g d.m.). The packaged samples had an average protein content higher than that of the retail-manufactured pasta. However, this higher value is due to the presence of sample B5 (protein = 20.16 g/100 g d.m.). Without this sample, the average protein content (14.82 g/100 g d.m.) would be very similar to that of the retail-manufactured samples.

The fat content of the packaged pasta samples, evaluated in terms of ether extract, was related in some way to the percentage of eggs declared on the label of each brand and was therefore highly variable. Samples B15 and B20 had the lowest values, which were below the legal limit (2.80 g/100 g d.m.). One case of non-compliance was also found among the samples of retail-manufactured pasta (sample RM2), also this group had a rather high intersample variability.

Only ten samples, all packaged pasta, contained detectable quantities of furosine. The products can be divided into three groups divided according to the furosine levels found: the first group is comprised of the samples with undetec-

Table 1 - Chemical characteristics of packaged (B) and retail-manufactured (RM) pasta samples.

Sample	Moisture (g/100 g)	Protein (g/100 g d.m.)	Lipid (g/100 g d.m.)	Furosine (mg/100 g protein)
B1org	27.98±0.06	14.34±0.17	3.96±0.08	n.d.
B2	29.90±0.09	13.46±0.06	3.63±0.03	n.d.
B3	29.39±0.13	12.73±0.15	2.91±0.06	18.00±0.84
B4	29.84±0.01	13.57±0.51	3.56±0.04	22.32±1.74
B5	29.43±0.01	20.16±0.30	6.15±0.07	n.d.
B6org	31.29±0.04	15.44±0.21	4.62±0.01	n.d.
B7	30.79±0.06	13.89±0.11	3.70±0.07	28.05±1.26
B8	27.90±0.10	13.73±0.04	2.98±0.01	n.d.
B9	31.86±0.03	14.94±0.46	3.29±0.13	n.d.
B10	27.15±0.07	16.69±0.08	3.09±0.08	33.75±2.50
B11	26.67±0.03	15.44±0.26	3.54±0.11	29.16±0.02
B12	31.11±0.13	15.10±0.14	2.93±0.02	5.84±0.14
B13	31.91±0.25	15.57±0.03	3.33±0.06	n.d.
B14	31.62±0.17	16.44±0.12	3.75±0.01	n.d.
B15	28.93±0.01	15.90±0.06	1.74±0.04	n.d.
B16	28.38±0.25	14.93±0.16	4.03±0.06	14.79±0.27
B17	30.93±0.09	14.90±0.21	3.42±0.04	7.38±0.43
B18	29.39±0.52	13.42±0.05	3.49±0.03	n.d.
B19	29.23±0.04	15.92±0.35	3.65±0.01	5.13±0.16
B20	30.75±0.10	15.20±0.02	2.41±0.02	6.03±0.59
Mean	29.72	15.09	3.51	17.05
CV%	5.25	10.66	24.80	63.76
RM1	28.88±0.16	14.23±0.23	3.97±0.13	n.d.
RM2	29.10±0.05	14.44±0.23	2.59±0.08	n.d.
RM3	29.72±0.07	15.56±0.04	3.10±0.06	n.d.
RM4	28.89±0.04	13.88±0.31	3.80±0.08	n.d.
RM5	29.07±0.03	13.77±0.16	3.07±0.05	n.d.
Mean	29.13	14.38	3.31	
CV%	1.18	4.97	17.22	

Each value is the mean±SD of two determinations; n.d.: not detectable; CV: coefficient of variation.

table levels of furosine (all retail-manufactured samples and 10 of the packaged samples), the second consists of products with a furosine content lower than 8 mg/100 g protein (4 packaged samples) and the third group is made of samples with a furosine content of 15 mg/100 g protein or more (6 packaged samples). RESMINI and PELLEGRINO (1991) reported a furosine content of between 10 and 120 mg/100 g protein for samples of retail-manufactured or packaged fresh egg pasta on sale to the public. These values are much higher than our own findings. As regards the packaged samples, these results could be due to the changes in production technologies used for fresh egg pasta and, in particular, to packing in a modified atmosphere, a process authorised since 1994 (DM, 1994), which gives the product a longer shelf life, even if the thermal treatment is milder.

Data related to the thickness of the raw pasta sheet and the cooking behaviour of all the samples of fresh egg pasta analysed are shown in Table 2. Packaged and retail-manufactured pastas had a very similar average thickness. Within the group of packaged products, sample B18 was distinctly different (the

Table 2 - Thickness and cooking behaviour of packaged (B) and retail-manufactured (RM) pasta samples.

Sample	Thickness [a] (mm)	Cooking behaviour	
		Matter loss [b] (g/100 g d.m.)	Weight increase [b] (%)
B1org	1.00±0.01	3.86±0.11	47.64±5.68
B2	1.37±0.03	2.00±0.05	25.75±1.65
B3	1.25±0.04	2.74±0.09	26.65±1.87
B4	1.01±0.01	2.84±0.04	32.56±1.85
B5	0.90±0.01	3.15±0.18	65.62±6.40
B6org	1.00±0.01	3.51±0.10	64.45±10.22
B7	1.01±0.01	2.75±0.02	34.31±0.21
B8	1.10±0.01	3.50±0.06	48.58±2.31
B9	1.01±0.01	3.75±0.07	40.23±3.56
B10	1.20±0.01	2.97±0.38	35.51±2.31
B11	1.20±0.01	2.66±0.22	28.46±2.59
B12	1.02±0.01	3.82±0.04	34.75±9.09
B13	0.98±0.03	4.66±0.14	67.03±1.28
B14	1.07±0.03	3.33±0.07	35.58±1.54
B15	1.01±0.01	4.30±0.46	39.37±4.85
B16	0.97±0.03	2.89±0.08	35.10±8.21
B17	1.02±0.01	2.69±0.52	29.29±2.14
B18	0.60±0.01	5.43±0.49	89.79±8.91
B19	1.01±0.01	3.91±0.07	35.26±4.53
B20	1.01±0.01	3.77±0.07	30.69±4.42
Mean	1.04	3.43	42.33
CV%	14.67	23.32	39.84
RM1	1.25±0.04	3.69±0.48	40.59±4.16
RM2	0.95±0.05	5.68±0.15	31.80±2.67
RM3	1.13±0.03	4.93±0.07	28.49±3.86
RM4	1.12±0.03	3.22±0.07	27.60±3.04
RM5	0.95±0.05	6.20±0.33	80.81±6.56
Mean	1.08	4.74	41.85
CV%	11.97	26.80	53.46

[a]Each value is the mean±SD of three determinations; [b]each value is the mean±SD of four determinations; CV: coefficient of variation.

thinness of the pasta sheet is one of its selling points) and sample B2, which was the thickest, is the only one sold as a rolled sheet.

The cooking behaviour of the various samples analysed, evaluated in terms of amount of matter loss and pasta weight increase, proved to be highly variable. This variability is explained by a set of factors, such as the characteristics of the flour mix used, the quantity of egg utilised (DALBON *et al.*, 1981; DALBON, 1983; D'EGIDIO *et al.*, 1983), and the thermal treatment that the pasta underwent. The thickness of the pasta sheet probably plays an important role: indeed, when considering the packaged samples, the lowest values for matter loss and weight increase were found in sample B2, which was the thickest sample, while the thinnest sample (B18) had the highest values. It should be noted that the cooking tests were carried out for a standard time and temperature (3

min, 100°C), even if this is not necessarily the ideal cooking time for all pasta samples.

The samples which had the highest matter loss were among the retail-manufactured pastas. These results could be due to the flour mix quality but, above all, to the fact that these pastas had had no thermal treatment. Pasteurisation leads to the denaturation of the proteins and the consequent stiffening of the structure, which in turn reduces the loss of solid matter in the cooking water (PAGANI *et al.*, 1999).

Fig. 1 shows the colour indexes and the influence of cooking on the colour of retail-manufactured and packaged pasta samples; the packaged pastas are divided into organic and non-organic pastas. The use of organic eggs for the production of organic pasta samples gives rise to a paler colour, as shown by the lowest a* and b* values. Although no sensory test was carried out, colour differences between raw retail-manufactured (paler) and raw non-organic packaged samples (darker yellow) were clearly visible to an observer. Cooking reduced both a* and b* indexes, turning all samples to a greener and less yellow colour.

Table 3 shows the results for the tensile tests carried out on the pasta sheets before and after cooking. The three parameters considered showed a very high intersample variability, attributable to the differences in the formulations used, the characteristics of the raw materials and the production process. A high intrasample variability was also recorded, probably caused by a lack of homogeneity in the structure of the pasta sheet. Indeed the greatest intrasample variability was found for the retail-manufactured raw samples, providing evidence that one result of retail-manufactured production processes is a less standardised product. Retail-manufactured fresh egg pasta had significantly higher break strain values than the packaged samples, even though its moisture levels were similar to those of the packaged group. This can be attributed to the absence of thermal treatment: pasteurisation leads to a significant increase in the firmness of the pas-

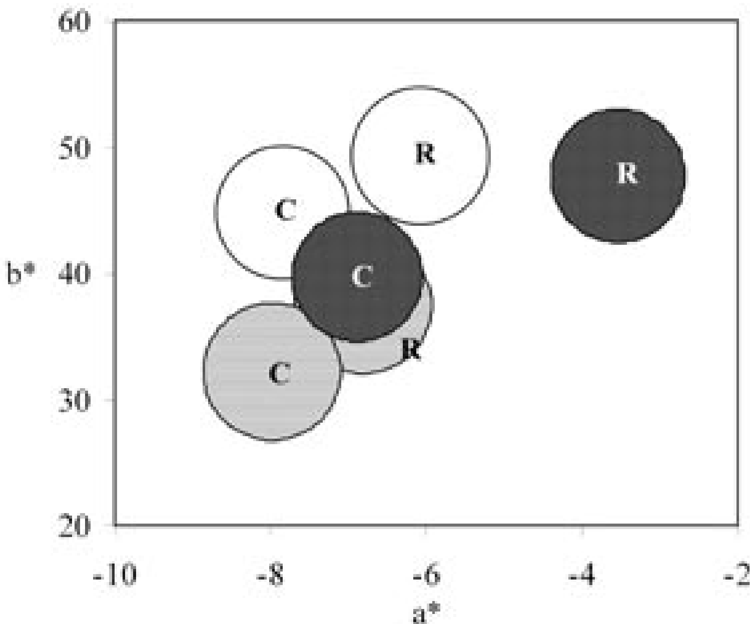

Fig. 1 - Colour indexes of packaged (○), packaged organic (◉) and retail-manufactured (●) raw (R) and cooked (C) pasta samples. a* and b* indexes represent the mean values of each class of samples; bubble diameter is proportional to L* parameter.

Table 3 - Rheological indexes of packaged (B) and retail-manufactured (RM) pasta samples before and after cooking.

Sample	Raw Pasta			Cooked Pasta		
	Break load (N)	Break strain (%)	Modulus (MPa)	Break load (N)	Break strain (%)	Modulus (MPa)
B1org	4.21±0.11	6.53±0.65	9.53±0.36	1.75±0.07	84.65±4.44	0.29±0.02
B2	7.22±0.54	9.30±0.93	7.86±1.18	3.73±0.20	82.51±11.13	0.57±0.05
B3	9.56±1.04	9.66±0.96	14.74±3.31	3.37±0.35	55.39±6.83	0.66±0.07
B4	9.00±0.39	12.29±1.15	13.17±1.60	3.42±0.13	74.83±8.95	0.53±0.03
B5	3.29±0.19	17.65±2.30	4.61±0.64	1.74±0.08	30.35±2.43	0.30±0.02
B6org	3.07±0.12	8.38±0.54	3.93±0.82	1.37±0.09	66.02±3.90	0.22±0.02
B7	8.73±0.46	6.32±0.40	13.18±0.17	2.24±0.21	35.99±4.19	0.51±0.05
B8	5.55±0.30	10.47±1.96	8.82±0.75	2.26±0.11	73.41±8.48	0.32±0.02
B9	6.77±0.16	13.64±1.17	5.47±0.39	2.05±0.07	67.24±9.39	0.29±0.01
B10	13.34±0.22	11.06±0.21	23.89±0.51	3.95±0.36	53.34±11.47	0.74±0.14
B11	12.24±0.77	10.22±1.06	17.80±1.12	3.97±0.50	59.23±2.02	0.68±0.12
B12	6.33±0.98	8.51±2.48	6.85±1.40	2.28±0.23	73.69±6.61	0.30±0.05
B13	6.58±0.78	11.43±1.09	6.81±0.86	1.80±0.18	65.18±5.23	0.24±0.02
B14	5.97±0.20	9.49±0.31	9.76±0.54	2.33±0.04	80.00±1.87	0.32±0.01
B15	7.18±0.31	10.58±0.57	10.54±1.58	2.11±0.05	65.45±6.97	0.33±0.02
B16	4.44±0.67	4.44±0.70	8.98±2.28	1.36±0.32	54.39±8.55	0.23±0.06
B17	6.11±1.23	7.74±1.41	10.78±1.74	2.34±0.13	64.14±9.18	0.41±0.09
B18	4.02±0.28	7.77±1.06	6.79±1.42	1.01±0.15	65.68±10.25	0.12±0.01
B19	4.72±0.47	8.11±1.97	8.37±0.80	2.02±0.16	74.18±4.11	0.27±0.04
B20	6.67±0.34	11.02±1.89	6.63±0.69	2.22±0.13	77.54±3.76	0.27±0.02
Mean	6.75	9.73	9.93	2.37	65.16	0.38
CV%	40.56	29.47	48.23	36.72	21.78	45.68
RM1	1.71±0.27	21.89±3.39	3.66±1.11	3.25±0.20	90.38±8.53	0.45±0.05
RM2	1.08±0.08	37.30±9.25	1.63±0.35	2.05±0.20	74.97±7.47	0.24±0.02
RM3	0.94±0.03	34.20±3.25	1.35±0.22	1.72±0.11	75.51±5.16	0.30±0.03
RM4	0.70±0.01	28.51±3.45	1.22±0.15	2.20±0.14	92.87±8.17	0.31±0.02
RM5	2.52±0.52	17.60±3.84	5.31±1.17	1.20±0.05	88.40±4.65	0.17±0.01
Mean	1.39	27.90	2.63	2.08	84.43	0.29
CV%	52.79	29.48	68.10	36.29	10.11	35.23

Each value is the mean±SD of at least six determinations; CV: coefficient of variation.

ta, accompanied by a drastic decrease in break strain (PAGANI *et al.*, 1999). Cooking endowed packaged pastas with a significant increase in break strain and a considerable decrease in the break load and Young's modulus, which indicate, respectively, the toughness and the hardness of the pasta. In the case of retail-manufactured pasta, cooking generally caused a drop in intersample variability. Unlike findings for packaged pastas, break load values increased after cooking (with the exception of sample RM5). In the loose pasta, cooking caused denaturation of proteins, leading to a more compact and firmer structure than that of raw pasta, while in the packaged pastas, which had already undergone thermal treatment and whose proteins had already been partly denatured, the effect of the gelatinisation of the starch probably prevails during cooking, together with that of water absorption, which makes the structure softer and more lax.

A number of significant correlations ($p<0.05$) between the variables were identified. In particular, break loads and Young's modulus, as evaluated on the raw pasta sheets, proved to be directly correlated ($p<0.001$) with the respective indexes measured after cooking. Moreover, modulus and load were directly correlated with one another ($p<0.01$), both before and after cooking. It can thus be affirmed that cooking changes these parameters proportionally to their initial values.

As can be observed in Fig. 2, the ratings obtained for Young's modulus, both for raw and cooked pasta sheets, were directly correlated with detectable levels of furosine ($p<0.01$), an index of the thermal damage that the pasta underwent. This indicates that as the intensity of the heat treatment increases, the structure of the pasta becomes stiffer and more compact. A further confirmation of this phenomenon comes from the fact that there was an inverse correlation between the matter loss in the cooking water and both the modulus ($p<0.01$) (Fig. 3) and furosine ($p<0.05$); if less matter is released during cooking, this indicates a more compact structure.

Matter loss and weight increase due to cooking were directly correlated ($p<0.001$): starch gelatinisation, a phenomenon which consists in the imbibition of the starch grains and their partial solubilisation, occurs during cooking (DALBON *et al.*, 1981; RESMINI *et al.*, 1988).

Multivariate analysis

Principal Component Analysis (PCA) was carried out using the data from all the samples analysed, in order to highlight which parameters had the greatest effect on the variability of the samples and identify any possible classifications. The results obtained for a packaged sample analysed at various times during storage at 4°C for 30 days were also included in the results. When the contribution of each variable to the total explained variance and the correlations

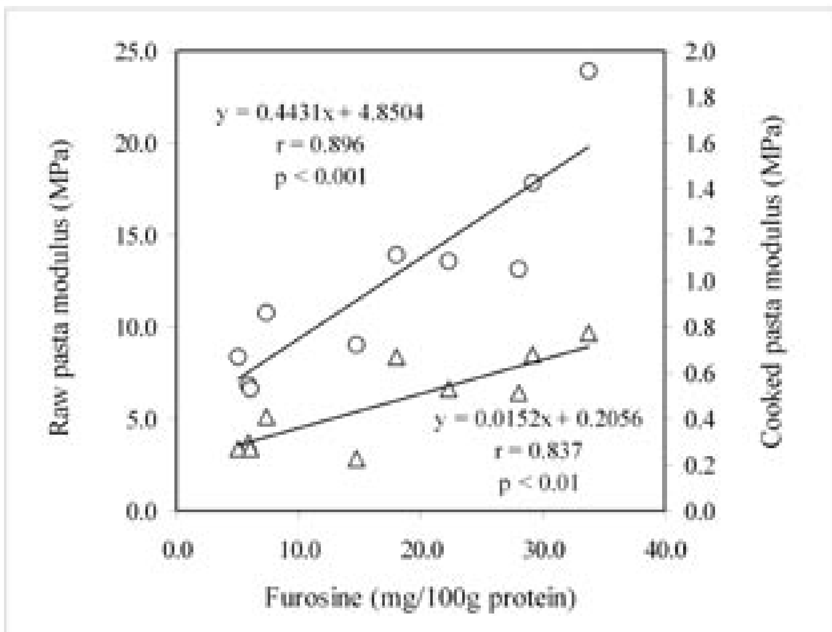

Fig. 2 - Linear correlation plot of Young's modulus of raw (○) and cooked (Δ) pasta samples *vs.* detectable furosine level.

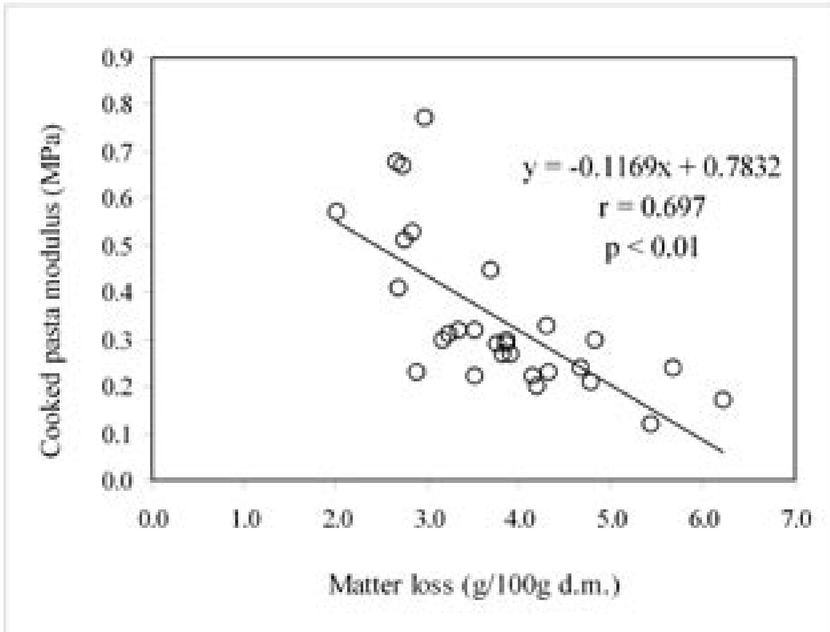

Fig. 3 - Linear correlation plot of Young's modulus of cooked pasta samples *vs.* matter loss.

between the variables were considered, it was possible to eliminate the variables that were less significant or which provided similar information. A chemometric model with ten variables was identified, which explained 65% of the total variance on the first two principal components (PC). Fig. 4 shows the projection of the loadings of the selected variables on the first two components. The break load of both raw and cooked pasta, matter loss, and furosine proved to have maximum loadings on the first PC. The colour indexes a* and L* and break strain, all of raw samples, explained a high percentage of the variation on the second PC.

The three groups can be identified in Fig. 5, which shows a projection of the sample scores on the first two components. These groups were further confirmed by Linear Discriminant Analysis, which correctly classified all samples.

A first group, differentiated on the first PC, includes pre-packaged pasta samples B2, B3, B4, B7, B10 and B11, which were characterised by the highest furosine values and break load, evaluated both on raw and cooked pasta, and by low matter loss. Indeed all the samples with furosine levels over 15 mg/100 g protein were found in this group, with the exception of sample B2, which had undetectable levels of furosine. This pasta was characterised by its greater thickness and high break load value of the cooked product. The other two groups were characterised by low or undetectable furosine and greater matter loss. In turn, they separate on the second PC on the basis of colour indexes (L* and a*) and the break strain of the raw pasta: the retail-manufactured samples make up a single group, which can be easily distinguished from the packaged samples.

The model clearly shows that on the first PC the samples are distributed according to the intensity of the thermal treatment applied, which affects the levels of furosine and the rheological and functional parameters. Indeed, the group of retail-manufactured pastas, which did

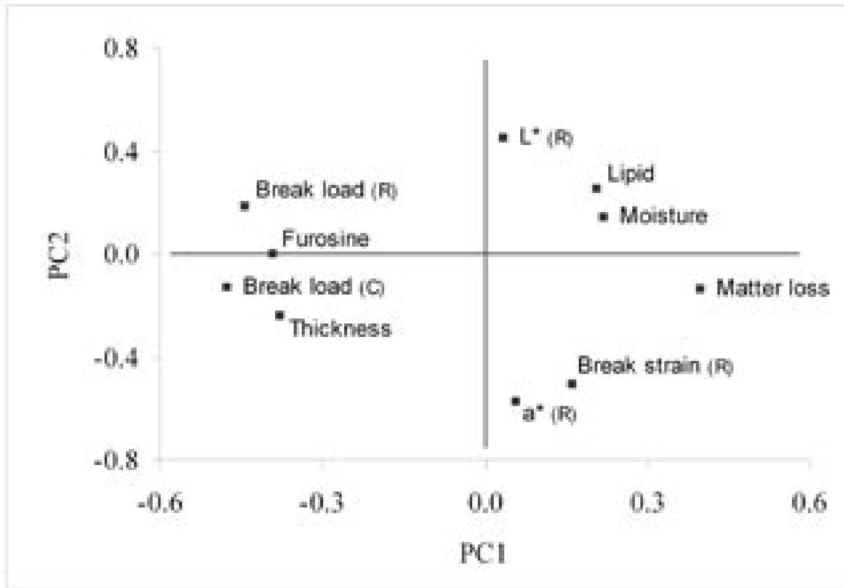

Fig. 4 - Loadings of selected variables on the first two principal components. (R) and (C) indicate if variable is evaluated on raw or cooked pasta, respectively.

not undergo any thermal treatment, contrasts sharply with the group containing the packaged samples with a high furosine content. The latter had probably been subjected to a double thermal treatment or one very intensive treatment, unlike the remaining packaged samples which are set in the right upper quadrant of Fig. 5. This assumption is supported by personal communications from several producers of the commercial brands analysed, who provided in-

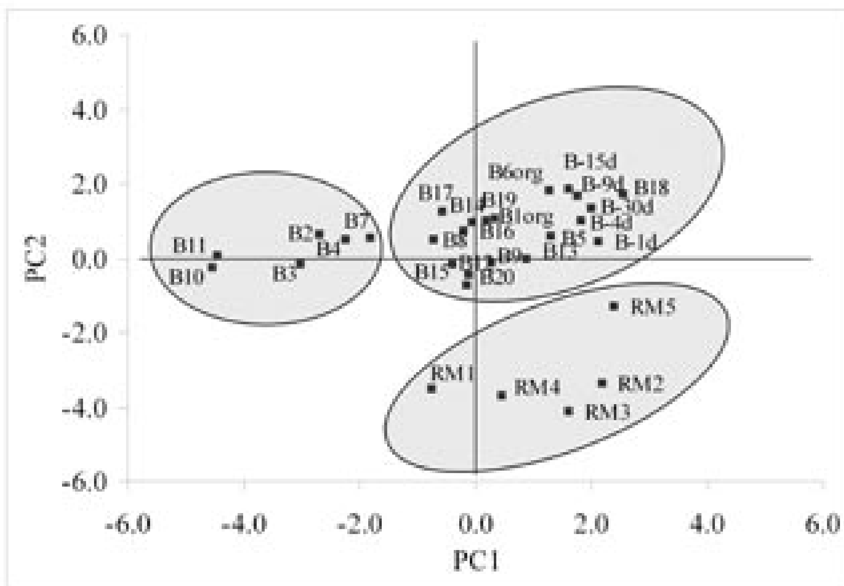

Fig. 5 - Scores of pasta samples on the first two principal components. B-1d, B-4d, B-15d, and B-30d identify a commercial brand of packaged samples analysed at various times during storage.

formation regarding the technology used in the production process and, in particular, the number and intensity of the thermal treatments applied.

CONCLUSIONS

This study revealed high variability in the composition and rheological and functional characteristics of fresh egg pastas on the market. The variability in the rheological and functional characteristics is attributable, above all, to the intensity of the thermal pasteurisation treatment(s) to which the pasta was subjected during the production process. The more severe the treatment, the tougher and harder the pasta becomes, while lower values were found for break strain and matter loss in the cooking water. Retail-manufactured pastas, which have had no thermal treatment, can be clearly distinguished from packaged samples. It was also observed that cooking the pasta causes an overall reduction in the variability of the rheological characteristics, making retail-manufactured and packaged pastas more similar to one another.

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PAPER

MULTIVARIATE SENSORY PROFILE OF BROCCOLI AND CAULIFLOWER AND CONSUMER PREFERENCE

ANALISI MULTIVARIATA DEL PROFILO SENSORIALE E PREFERENZE DEI CONSUMATORI SU BROCCOLI E CAVOLFIORI

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ABSTRACT

Sensory profiles were set up for green, violet and Chinese broccoli cultivars and white, green, pyramidal and purple cauliflower cultivars, which reflect a wide range of products available on the market. A trained panel of ten judges differentiated almost all the samples based on most of the 33 attributes tested. Consumer panel members preferred cauliflower samples with greater sweetness, juiciness and cauliflower flavour. Sweetness, crispness and intensity of broccoli and cauli-

RIASSUNTO

Sono tracciati i profili sensoriali di alcune cultivar di broccoli (verdi, viola e cinesi) e di cavolfiori (bianchi, verdi, viola e a forma di piramide), scelte in quanto rappresentative di un'ampia gamma di prodotti presenti nel mercato. Il panel, composto da 10 giudici addestrati, ha indicato le differenze esistenti tra quasi tutti i campioni servendosi della maggior parte dei 33 descrittori esaminati. Dall'analisi effettuata è emerso che i consumatori, membri del panel group, preferiscono cavolfiori più

- Key words: Acceptability, bitterness, broccoli, cauliflower, consumer preference, sensory profile -

flower flavour were the most important attributes for broccoli acceptability. More intense bitter, pungent and green/grassy notes reduced acceptability. The calculation of individual response to the product attributes revealed substantial differences between the individual consumers. A large majority of consumers indicated a greater preference for both, broccoli and cauliflower samples which had more sweetness and cauliflower flavour and low intensities of bitter and pungent notes. While sugar content could not be used to predict sweetness, glucosinolate content affected sweetness as well as bitter and pungent notes.

dolci, succosi e con un più spiccato aroma di cavolfiore. Sapore dolce, croccantezza e un intenso aroma di broccoli e cavolfiore sono fattori importanti per l'accettabilità del prodotto da parte del consumatore; viceversa un intenso gusto amaro, pungente ed erbaceo ne riduce l'accettabilità. La valutazione individuale dei giudizi ha evidenziato una notevole differenza tra le risposte dei singoli, ma la maggior parte dei consumatori ha espresso parere favorevole per broccoli e cavolfiori con uno spiccato gusto dolce e aroma di cavolfiore ed un limitato gusto amaro e pungente. Infine si è riscontrato che il contenuto in zucchero non influisce sulla dolcezza del campione, ma è il glucosinolato che conferisce il gusto dolce, l'amaro e le note pungenti al prodotto.

INTRODUCTION

Following a decade of a rapid increase in the production and consumption of broccoli in Europe, sales have begun to stagnate in the last years. The consumption of cabbage and the production of cauliflower also decreased in the EU in the same period (BEHR and ILLERT, 2002). Producers are trying to counteract this trend, by diversifying, for example, by introducing coloured cultivars.

Positive health effects of broccoli and cauliflower can be attributed to individual glucosinolates (WATZL and LEITZMANN, 1999, ZHANG *et al.*, 1994, DILLARD and GERMAN, 2000; VERHOEVEN *et al.*, 1997), but the effect that these substances have on the flavour is less clear (BUTTERY *et al.*, 1976; HANSEN *et al.*, 1997; BAIK *et al.*, 2003). The levels of specific glucosinolates were found to be associated with sensory attributes in Brussels sprouts cultivars (FENWICK *et al.*, 1983, 1990; VAN DOORN *et al.*, 1998), green broccoli (HANSEN *et al.*, 1997) and cauliflower (ENGEL *et al.*, 2002). Some of the glucosinolates have bitter tastes, which are frequently disliked and are one reason for low acceptability of Brassica species (DREWNOWSKI and GOMEZ-CARNEROS, 2000; SCHONHOF *et al.*, 2004). Differences in the acceptability of cauliflower cultivars were found to be caused by two glucosinolates and other sulphur-containing substances (ENGEL *et al.*, 2002). Very little is known about the composition of coloured cauliflower and the resulting sensory attributes (LEWIS *et al.*, 1991).

The aim of this work was to set up sensory profiles for a variety of green, violet and Chinese broccoli cultivars as well as white, green, pyramidal and purple cauliflower cultivars reflecting a wide range of the market diversity. To improve the validity of the results, the influence of three different years of cultivation were also included, because it has been shown, that the growing conditions have a substantial influence on

the glucosinolate content (FARNHAM, 2004; BROWN, 2002).

The relationship between the sensory profiles and consumer acceptability were investigated. Potential differences in consumer preferences for sensory attributes, already known for other commodities were examined for broccoli and cauliflower. The results of the study could be used for product improvement and to enable consumer oriented marketing decisions. Diversification and cultivar improvement for the production of winter broccoli would be beneficial, because sales in Germany are concentrated in the winter months whereas other sites of European winter production are limited to Murcia and Valle del Ebro in Spain and Puglia in Italy.

MATERIALS AND METHODS

Plant material and growing conditions

In three consecutive years (1998, 1999, 2000) different members of the Brassica family were grown in the open field on sandy soil in Grossbeeren near Berlin. Fertilisation, irrigation and determination of harvest maturity were conducted according to integrated horticultural practices (WINKHOFF, 1992).

The green broccoli (*Brassica oleracea* var. *italica*) cultivars Emperor, Marathon, Shogun and the violet cultivar Viola were grown. A Chinese species (*Brassica rapa* var. *alboglabra*) with loose florets was also included. The cauliflower (*Brassica oleracea* var. *botrytis*) samples included Rosalind, a purple cultivar, the green cultivar Minarett with a typical, pyramidal shaped inflorescence and Marine, a conventional white cultivar. The crops were harvested in October in each of the three years. Instrumental measurements of compounds were conducted according to Schonhof (2004) and are reported as fresh mass (FM) of plant material.

Sensory analysis and consumer panel

The sensory tests were conducted by means of quantitative descriptive analysis related to QDA® according to Stone and Sidel (1993), using profiling of a trained panel. Ten judges were selected, who had successfully passed standardised tests for olfactory, taste and colour sensitivity as well as memory, verbal ability and creativity. The judges participated in approximately ten weeks of training (one to two lessons per week) to establish the methodological foundations, followed by another ten weeks (one to two lessons per week) of working with the actual products. The attributes were described and the diversity and variability were ascertained. The descriptors were developed upon agreement of all the panelists. After eliminating any redundancy, rough profiles were established. Next, standards were developed, reliability testing was conducted and the results were validated. The product profiles were then used for testing. Table 1 presents the selected descriptors, their definitions and the material used as standards for the training. In each year, several lessons were given before the test to refresh the performance of the panelists.

Freshly harvested material was cut into florets with adherent second order stems. The material was presented uncooked, because it is often consumed raw for salads and could potentially be added as nutraceutical additives to other foods. The samples were presented individually and in random order. The intensity evaluation of the sensory perception by the trained panel was carried out, using unstructured line scales with the anchor points 0 – not perceptible and 100 – strongly perceptible.

Both the trained judges and the consumer panelists worked in a sensory laboratory under defined conditions (temperature, light) in individual booths with computer equipment. The data acquisition was carried out using the CASA

Table 1 - Sensory descriptors and the assigned definitions and anchor points.

Descriptors		Definitions and anchor points
Taste	Bitter	Basic taste; stimulated by caffeine solutions
	Sweet	Basic taste; sucrose solutions
Flavour	Broccoli	Specific broccoli flavour note, selected samples
	Cauliflower	Specific cauliflower flavour note, selected samples
	Green/grassy	Fresh cut grass or unripe apples, trans-hexenal/cis-hexenol
	Pungent	Perception of painful burning/biting; capsaicine
Mouthfeel	Crisp	Force necessary for sudden break; apple, fresh and stored broccoli
	Juicy	Liquid producing sensation during chewing; apple pieces
Aftertaste	Bitter	Basic bitter taste perceptible after swallowing
	Pungent	Lasting perception of painful burning/biting

(Computer Aided Sensory Analysis) software.

For the sensory consumer test, representative groups of 62 (1998), 98 (1999) and 99 (2000) housewives, the target group of private family households, were selected from the general public. The selection criteria for the consumers were, that they were: (1) regular buyers of cauliflower and broccoli; (2) between 25 and 65 years of age; and (3) mainly responsible for selecting and purchasing the household food. They judged the acceptability of: first impression, external and internal appearance, odour, flavour, aftertaste, mouthfeel and overall impression. The acceptability of flavour, which was highly correlated with the overall impression (r=0.96) is reported. Unstructured line scales were used with the anchor points 0 – very unpleasant and 100 – very pleasant.

Data analysis

Consumer data were standardised to mean zero and standard deviation 1 for factor and cluster analysis. For a graphic depiction, Principal Component Analysis (PCA) was performed. The results are unrotated and the first two factors are shown. All calculations and contour plots were performed with the Statistica for Windows program (version 4.1, Statsoft Inc.) and SPSS (version 7.5).

RESULTS AND DISCUSSION

Sensory profiles

Broccoli and cauliflower samples were differentiated by almost all of the tested attributes. The results of the analysis of variance and F-test (Table 2) for taste, flavour, mouthfeel and aftertaste attributes showed significant differences with respect to the samples and the year of cultivation. A large degree of variation could be attributed to the judges, whereas the F-ratios of the interaction of judges*samples, judges*years and samples*years were lower. Further analysis showed, that sweetness separated the samples significantly ($p<0.001$) in 1998 and 2000. Nine other attributes also differentiated the samples significantly ($p<0.001$) in each of the three years.

Within the samples, important differences were related to the typical broccoli and cauliflower notes, which were assigned to the respective groups of broccoli and cauliflower samples. The flavour of the cauliflower-type "Rosalind" was perceived to be closer to broccoli, than to cauliflower.

Bitter taste and aftertaste notes were more intense in the "Shogun" and Chinese broccoli samples, while they were less intense in "Marine" cauliflower, which had a more prevalent sweet taste.

Table 2 - F-ratios and corresponding significance levels for the descriptors.

		Sample	Year	Judge	J*S	J*Y	S*Y
Taste	Bitter	22.07 ***	28.98 ***	7.26 ***	1.02 n.s.	1.18 n.s.	3.60 ***
	Sweet	6.96 ***	37.58 ***	8.07 ***	1.79 *	6.09 ***	1.59 n.s.
Flavour	Broccoli	32.3 ***	53.14 ***	3.56 **	0.99 n.s.	1.54 n.s.	0.82 n.s.
	Cauliflower	55.78 ***	96.13 ***	6.33 ***	0.71 n.s.	3.52 **	3.87 ***
	Green/grassy	8.86 ***	6.06 ***	13.45 ***	0.81 n.s.	8.40 ***	0.97 n.s.
	Pungent	7.27 ***	11.83 ***	5.55 ***	0.64 n.s.	1.87 n.s.	1.47 n.s
Mouthfeel	Crisp	9.62 ***	3.12 *	9.59 ***	0.74 n.s.	4.18 ***	0.52 n.s.
	Juicy	10.19 ***	8.77 ***	14.97 ***	1.58 n.s.	9.55 ***	1.99 *
Aftertaste	Bitter	13.36 ***	46.24 ***	2.40 n.s.	1.60 n.s.	2.71 **	2.87 **
	Pungent	9.85 ***	36.22 ***	5.37 **	1.44 n.s.	4.01 ***	2.38 **

F-ratios marked with asterisks indicate significance at: * p<0.05, ** p<0.01, *** p<0.001; n.s. no significant difference.
J*S = judges x samples; J*Y = judges x years; S*Y = samples x years.

Besides the differences in the broccoli and cauliflower cultivars, a substantial variation in several attributes was attributable to the conditions during the three years of cultivation. Bitter taste and aftertaste were significantly more intense in 1998 and 1999, compared to 2000, whereas sweet taste and many flavour attributes were the lowest in 1998 (Tables 3-5).

Sensory dimensions

The multivariate structure of the sensory space was analysed by performing Principal Component Analysis (PCA). The results of the sensory attributes, which significantly differentiated the flavour, mouthfeel and aftertaste attributes were calculated. The first factor extracted explains 56% of the variation of the senso-

Table 3 - Mean sensory scores of the quantitative, descriptive analysis of broccoli and cauliflower in 1998.

		Green Broccoli			Chinese Broccoli	Cauliflower			
	1998	Emperor	Marathon	Shogun		Viola	Rosalind	Minarett	Marine
Taste	Bitter	49 cd	21 a	59 d	60 d	48 bcd	41 bc	37 b	23 a
	Sweet	9 a	14 ab	9 a	17 b	12 ab	9 a	17 b	26 c
Flavour	Broccoli	49 b	48 b	47 b	25 a	45 b	41 b	40 b	16 a
	Cauliflower	10 a	11 ab	12 ab	9 a	10 a	23 c	18 bc	62 d
	Green/grassy	36 bc	30 ab	40 cd	45 d	33 bc	32 bc	36 bc	22 a
	Pungent	40 c	25 a	42 c	42 c	42 c	43 c	36 bc	28 ab
Mouthfeel	Crisp	49 a	51 ab	46 a	45 a	55 ab	48 a	63 bc	69 c
	Juicy	25 b	24 a	29 ab	38 c	27 ab	27 ab	33 bc	48 d
Aftertaste	Bitter	47 cd	26 a	50 cd	53 d	42 bc	33 ab	33 ab	24 a
	Pungent	39 bcd	26 a	45 cd	45 d	36 abc	34 ab	38 bcd	31 ab

Means within a row with different subscripts are significantly different at p<0.05.
Rating scale ranged from 0 (not perceptible) to 100 (strongly perceptible).

ry attributes, while the second factor accounts for another 20% of the variation in the data set (Fig. 1). These two factors were the only factors that had Eigenvalues above 1.

Negative loadings of the first factor are defined by sweet taste, crisp mouthfeel and cauliflower flavour. Green/grassy and pungent flavour and bitter taste were associated and resulted in positive loadings. The second factor separated broccoli flavour and juicy mouthfeel.

In the score plot (Fig. 2) the samples are partly separated according to the year of cultivation. Samples grown in 1998 are shifted to the upper right quadrant, indicating less sweetness and increased bitter and pungent notes. The

Table 4 - Mean sensory scores of the quantitative, descriptive analysis of broccoli and cauliflower in 1999.

		Green Broccoli					Cauliflower		
	1999	Emperor	Marathon	Shogun	Chinese Broccoli	Viola	Rosalind	Minarett	Marine
Taste	Bitter	34 b	38 b	48 cd	51 d	42 bc	43 bcd	38b	20 a
	Sweet	28 b	26 ab	23 ab	25 ab	18 a	18 a	27ab	26 b
Flavour	Broccoli	62 c	63 c	63 c	47 b	57 c	57 c	57c	23 a
	Cauliflower	28 a	32 ab	24 a	23 a	29 a	27 a	41b	63 c
	Green/grassy	35 abc	35 abc	44 c	43 c	32 ab	29 ab	39bc	25 a
	Pungent	35 b	34 b	45 c	48 c	47 c	42 bc	36b	25 a
Mouthfeel	Crisp	50 a	58 ab	55 ab	51 a	58 ab	50 a	63bc	68 c
	Juicy	21 a	30 abc	29 abc	38 c	26 ab	23 a	30abc	37 bc
Aftertaste	Bitter	32 ab	34 ab	43 bc	48 c	42 bc	47 c	34ab	25 a
	Pungent	26 ab	41 cde	47 de	47 e	42 cde	37 cd	34bc	24 a

Means within a row with different subscripts are significantly different at p<0.05.
Rating scale ranged from 0 (not perceptible) to 100 (strongly perceptible).

Table 5 - Mean sensory scores of the quantitative, descriptive analysis of broccoli and cauliflower in 2000.

		Green Broccoli					Cauliflower		
	2000	Emperor	Marathon	Shogun	Chinese Broccoli	Viola	Rosalind	Minarett	Marine
Taste	Bitter	32 bc	29 b	29 b	48 d	30 b	27 b	37 c	17 a
	Sweet	27 a	28 a	28 a	25 a	26 a	22 a	23 a	38 b
Flavour	Broccoli	64 d	67 d	66 d	49 b	63 d	56 c	46 b	31 a
	Cauliflower	35 b	37 b	31 ab	26 a	35 b	37 b	56 c	65 d
	Green/grassy	38 c	34 bc	38 c	38 c	36 c	27 ab	32 bc	23 a
	Pungent	31 ab	30 ab	25 a	43 c	32 ab	36 bc	33 b	24 a
Mouthfeel	Crisp	49 ab	55 b	53 b	44 a	54 b	52 b	67 c	65 c
	Juicy	27 abc	31 abc	27 abc	34 c	29 abc	24 a	26 ab	34 bc
Aftertaste	Bitter	24 b	24 b	22 b	38 c	25 b	24 b	30 b	14 a
	Pungent	25 a	26 a	24a	39 b	27 a	28 a	27 a	22 a

Means within a row with different subscripts are significantly different at p<0.05.
Rating scale ranged from 0 (not perceptible) to 100 (strongly perceptible).

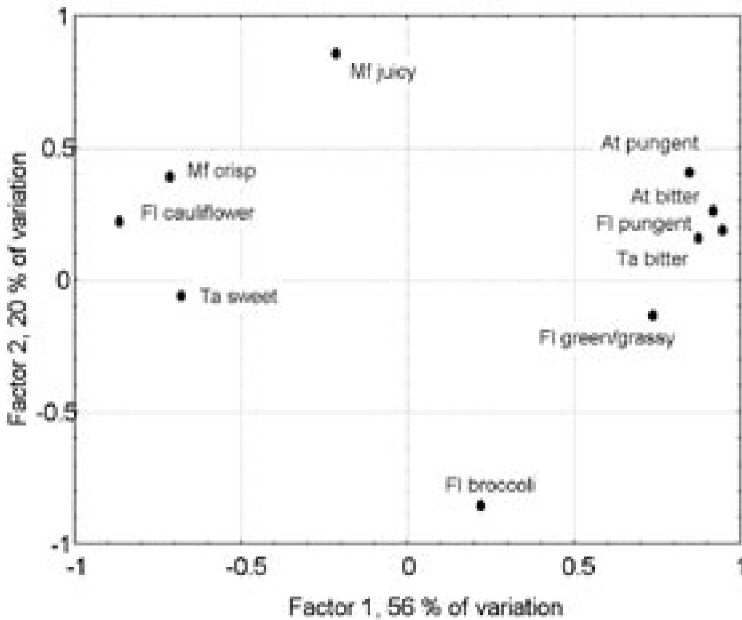

Fig. 1 - Loading plot of the sensory space formed by the combined quantitative, descriptive data from 1998, 1999 and 2000. At=aftertaste, Fl=flavour, Mf=mouthfeel, Ta=taste.

samples from 1999 are in an intermediate position, while the samples from 2000 are located on the lower part (negative) of the second factor, closely associated with a broccoli-like flavour, which was more intense in that year.

The white "Marine" cauliflower samples were characterised by a sweeter

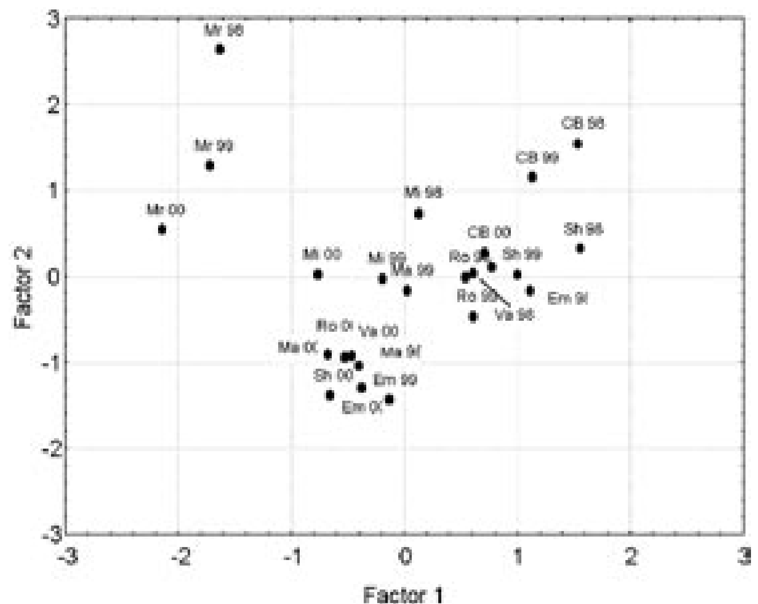

Fig. 2 - Score plot of the products sensory space formed by the combined QDA data from 1998, 1999 and 2000. CB=Chinese broccoli, Em=Emperor, Ma=Marathon, Mr=Marine, Mi=Minarett, Ro=Rosalind, Sh=Shogun, Va=Viola.

taste, cauliflower-like flavour and crisp mouthfeel. The Chinese broccoli, and to some extent "Shogun", produced bitter, pungent and green/grassy notes. The cauliflower type "Minarett" exhibits an intermediate position, while "Rosalind", another cauliflower-type did not differ from the broccoli samples. "Marathon" was characterised by a more broccoli-like flavour, whereas "Shogun" tended to have more pungent and bitter notes.

Sweetness and sugar content

The sweet taste intensities were influenced by the year of cultivation (14 to 27 units on average for all the cultivars) and by the samples of the broccoli and cauliflower cultivars (16 to 31 units on average for the three years). Sugar content also differed with the year (1.76 to 1.96 g/100 g FM on average for all cultivars) and with the cultivars (1.26 to 2.45 g/100 g FM on average for the three years). A higher sugar content did not coincide with increased intensity of sweet taste (R^2=0.01).

The distribution of sugar content is depicted in Fig. 3 based on the factor scores for all samples. In addition, the sugar content of the samples is superimposed as contour lines. Sweet taste, which was one of the notes that differentiated the products was not due to the sugar content, only. For example, "Marine" samples from the 2,000 crop, which had a sweet taste, had an average sugar content of 2 g/100 g FM. This was almost the same amount as that of "Shogun" or "Chinese" broccoli samples that were the least sweet and had the most intense bitter and pungent notes. These notes may have masked sweetness.

In contrast, samples with a low sugar content, such as "Emperor" from 1999 and 2000 did not have any correlation with sweetness.

Bitter and pungent notes

Bitter and pungent notes differed substantially among the samples. The lowest intensities were detected in the "Marine" cauliflower samples (20 and 26 units for bitter and pungent flavour, repectively on average for all the years). The most intense bitter and pungent notes were found in Chinese broccoli and "Shogun"

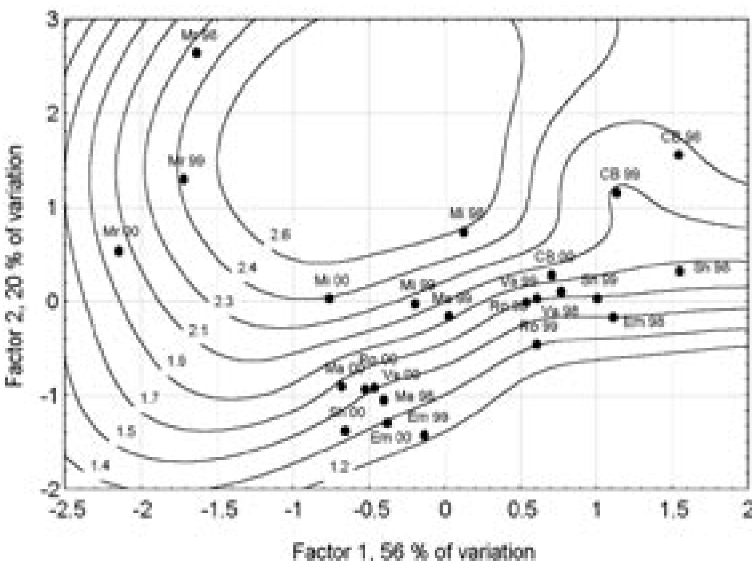

Fig. 3 - Score plot of the products with contour plot of the sugar content (g/100 g FM) of the samples. CB=Chinese broccoli, Em=Emperor, Ma=Marathon, Mr=Marine, Mi=Minarett, Ro=Rosalind, Sh=Shogun, Va=Viola.

(46-52 and 37-44 units for bitter and pungent flavour, repectively on average for all the years). These samples, as well as other broccoli samples grown in 1998 and some in 1999, are located on the right side of the score plot (Fig. 2). Harsh or bitter notes were partly associated with increased levels of total glucosinolates (FENWICK *et al.*, 1983, 1990; HANSEN *et al.*, 1997; ENGEL *et al.*, 2002) To identify a potential relationship between the bitter and pungent notes and the total glucosinolates, their concentrations were determined and reported by SCHONHOF *et al.* (2004). The amount of total glucosinolates was calculated from the sum of alkyl glucosinolates (glucoraphanin, glucoibervein, glucoerucin, glucoibervein), alkenyl glucosinolates (progoitrin, sinigrin, gluconapoleiferin, gluconapin), indole glucosinolates (glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin) and the aryl glucosinolate gluconasturtiin. The contents of each sample were projected onto the factor score plot (Fig. 4).

Because of the high diversity in the absolute contents of the glucosinolates, the response surface lines were calculated on a logarithmic basis. The highest glucosinolate values (> 400 mg/100 g FM), due to the Chinese Broccoli samples from 1998, are located in the upper right portion of the score plot. "Shogun" from the 1998 and Chinese broccoli from the 1999 season also contained high levels of total glucosinolates. These samples had the most intense pungent and bitter notes. Low values for bitter and pungent notes were measured in the "Marine" samples as well as in "Emperor" samples of 1999 and 2000. In these samples total glucosinolate concentrations were below 20 mg/100 g FM. SCHONHOF *et al.* (2004) reported that the total glucosinolate content is very strongly influenced by the daily mean temperatures, low temperatures, such as those in 1998, lead to elevated glucosinolate levels.

Consumer flavour preferences

The consumers clearly distinguished among the samples (Table 6). The highest acceptability was attributed to "Marine" cauliflower samples which received significantly higher scores throughout all three years. Among the broccoli sam-

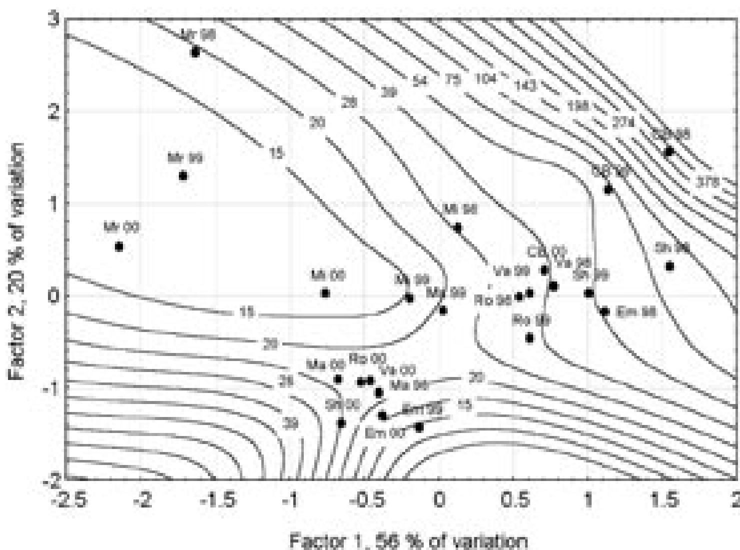

Fig. 4 - Score plot of the products with contour plot of the glucosinolate content (mg/100 g FM) of the samples. CB=Chinese broccoli, Em=Emperor, Ma=Marathon, Mr=Marine, Mi=Minarett, Ro=Rosalind, Sh=Shogun, Va=Viola.

Table 6 - Mean consumer acceptance scores for broccoli and cauliflower.

		Green Broccoli			Chinese Broccoli	Viola	Cauliflower		
		Emperor	Marathon	Shogun			Rosalind	Minarett	Marine
Overall liking									
	1998	61 a	75 c	62 ab	65 ab	72 bc	63 ab	76 c	86 d
	1999	76 d	73 d	63 bc	40 a	65 b	71 cd	75 d	87 e
	2000	68 a	69 a	-+)	-+)	65 a	-+)	69 a	70 b

Means within a row with different subscripts are significantly different at $p<0.05$.
+) not analysed.
Rating scale ranged from 0 (very unpleasant) to 100 (very pleasant).

ples, "Marathon" was liked the most, but had a lower score in 2000; this was also the case with "Marine". "Minarett" and "Emperor" had an intermediate position. "Shogun", "Viola", "Rosalind" and Chinese broccoli were liked the least.

In order to relate the product attribute intensities to the acceptance scores, these values were projected onto the factor score plot (Fig. 5).

The lowest acceptance values are located in the upper right position, defined by the pungent and bitter notes of Chinese broccoli and "Shogun" in 1998. An intermediate position with acceptance values between 65 and 75 characterised the 1999 and 2000 samples. These samples were also judged to have intermediate intensities of bitter, pungent and sweet notes. The "Marine" samples, with a sweet and cauliflower-like flavour, had the highest values.

To relate the intensities of each of the attributes to flavour acceptance, a Pearson correlation was calculated, separately for the broccoli and cauliflower sam-

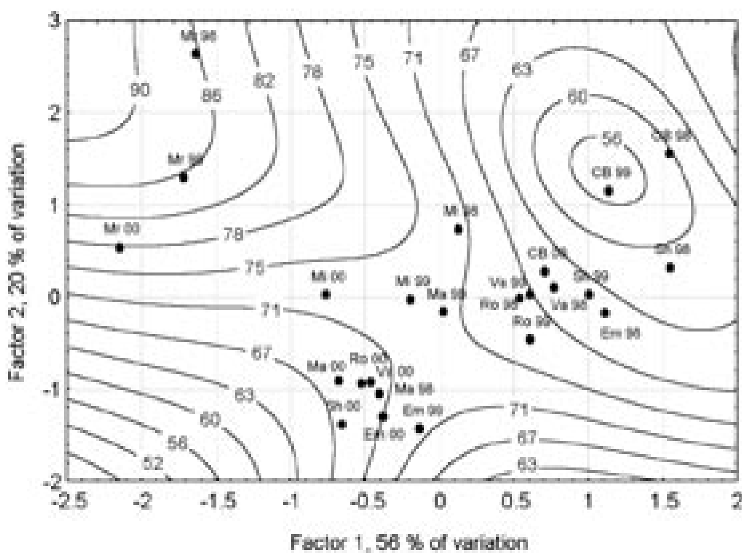

Fig. 5 - Score plot of the products with contour plot of the mean consumer acceptance scores of the samples. CB=Chinese broccoli, Em=Emperor, Ma=Marathon, Mr=Marine, Mi=Minarett, Ro=Rosalind, Sh=Shogun, Va=Viola.

ples. An association was found between sweet taste, juiciness and cauliflower flavour intensity and the preference for cauliflower samples (Fig. 6). A close negative correlation existed between flavour acceptance and bitter taste and aftertaste, pungent flavour and aftertaste and broccoli-like flavour.

Acceptability of the broccoli samples (Fig. 7) improved as sweetness and cauliflower-like flavour intensity increased along with broccoli flavour and crispness. Increased juiciness did not increase acceptance within the broccoli samples. A possible explanation could be, that Chinese broccoli samples had the highest juiciness values, but were less acceptable because of their intense bitter and pungent notes. Bitter taste, green/grassy and pungent flavour and aftertaste were closely and negatively correlated with acceptability.

The negative effect of bitter and/or pungent notes in vegetables has been well documented (DREWNOWSKI and GOMEZ-CARNEROS, 2000). The sensitivity to and acceptance of bitter notes varies (GREENHOFF and MACFIE, 1994; MOSKOWITZ, 2002; DREWNOWSKI *et al.*, 2000) and depends on individual preferences for flavours and tastes (PANGBORN, 1981; TUORILA, 1996). Consumers with different preferences can be grouped into consumer segments. In a study with apples, conducted by DAILLANTSPINNLER *et al.* (1996), consumers

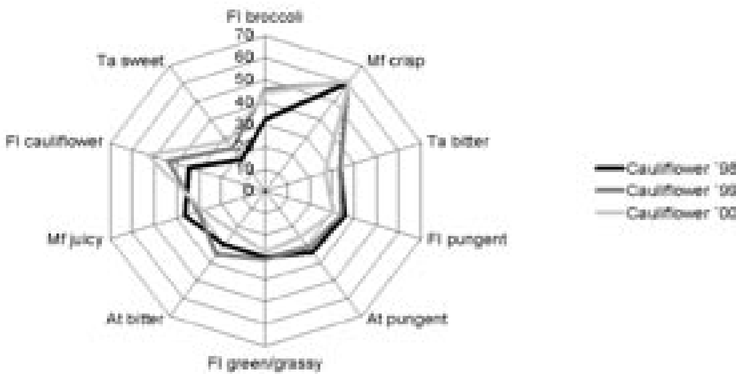

Fig. 6 - Cauliflower samples - Spearman correlation coefficients for the relation of product attributes and consumer acceptability. At=aftertaste, Fl=flavour, Mf=mouthfeel, Ta=taste.

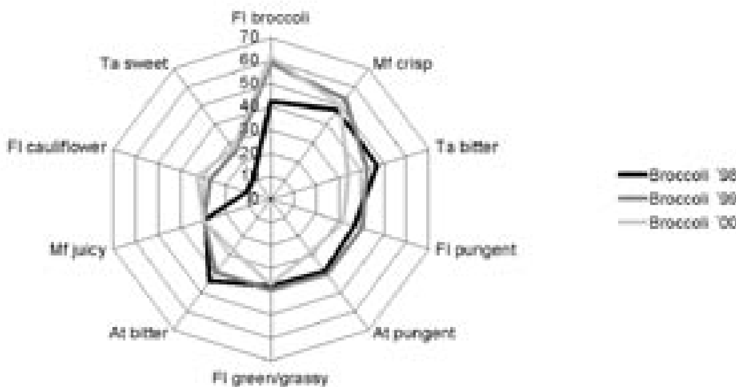

Fig. 7 - Broccoli samples - Spearman correlation coefficients for the relation of product attributes and consumer acceptability. At=aftertaste, Fl=flavour, Mf=mouthfeel, Ta=taste.

were classified according to preferences for a sweet, hard apple or a juicy, acidic apple. PAGLIARINI *et al.* (2001) reported two distinct consumer groups based on preferences for different tomato cultivars. The first group preferred cultivars that were red and sweet, while the second group preferred fruit that was acidic and had certain texture characteristics. Similar results were also found by BRÜCKNER (2000) and BRÜCKNER and AUERSWALD (2000). Knowledge about specific attributes, which underlie preferences within a target group, is required for product development. One example of product development with an altered set of attributes is the launching of a new yellow-fleshed, sweet, fruity flavoured cultivar of kiwifruit on the marketplace (JAEGER *et al.*, 2003). In this study, a comparison of consumer preferences for this new type of kiwifruit with that of the familiar green-fleshed and sweet-tart taste was undertaken. A segment of the population liked the yellow-fleshed kiwifruit genotypes. Despite their large sensory differences no such information is available for broccoli and cauliflower cultivars (PARADIS *et al.*, 1996; HANSEN *et al.*, 1997; GILLIES *et al.*, 1997; BAIK *et al.*, 2003; SCHONHOF *et al.*, 2004).

In an attempt to gain such information, the relationship between the attribute intensity and the individual consumer's score for flavour acceptance was determined (Fig. 8). A 25 and a 75 percentile of the correlation coefficients were calculated. This means, that 25% of all consumers had a lower correlation coefficient than marked by the vertical bars, and another 25% of the consumers had a higher coefficient than marked by the bars. The bars themselves mark the range of coefficients covered by the remaining 50% of the consumers.

When the attribute intensities of both broccoli and cauliflower samples were included, a large span of intensities was present and a clear separation between those attributes which are positively related to flavour acceptance and those which are negatively related became evident. An increase of bitter and pungent notes and some green/grassy and typical

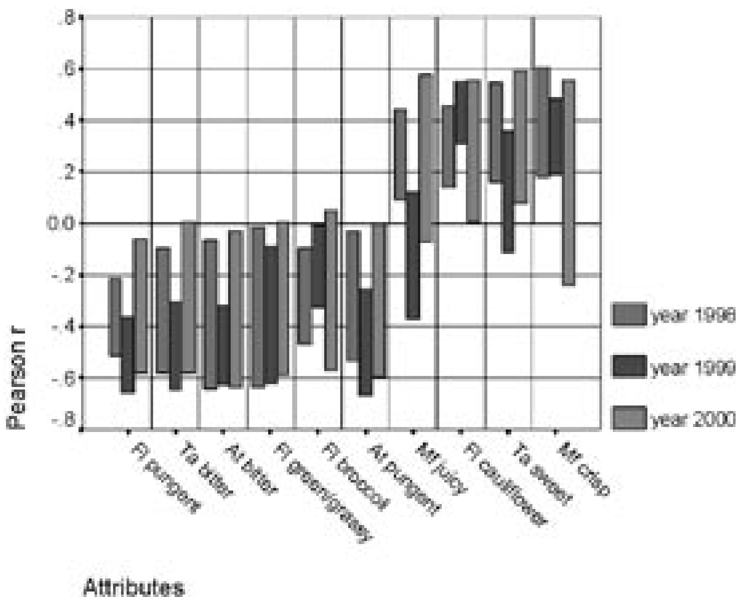

Fig. 8 - Distribution of the correlation coefficients for the relation of product attributes and individual consumer acceptability. Cauliflower and broccoli samples. At=aftertaste, Fl=flavour, Mf=mouthfeel, Ta=taste.

broccoli flavour lowered the acceptability for more than 75% of the consumers.

Crisp mouthfeel, sweet taste and cauliflower flavour were attributes which led to increased acceptance for the majority of the consumers. In 1999, the role of juiciness was not clear; this coincides with the fact that texture properties might attract little awareness within an expected range (SZCZESNIAK and KAHN, 1971).

To identify attribute combinations which promote acceptance, a further differentiation of the consumers was conducted by cluster analysis (MEILGAARD *et al.*, 1991). The separation was done on the basis of the correlation coefficients of the attribute intensities and the resulting individual flavour acceptance. Table 7 shows the average correlation coefficients of the consumer clusters which were separated by cluster analysis. Based on the average for all three years, members of cluster 1 did not accept increased bitter taste or pungent flavour. Crisp mouthfeel was important in this group, whereas the role of sweetness differed. Sweetness was the most important factor for acceptability for the members of the second consumer cluster, while bitter and pungent notes were disliked the most. In the third and smallest cluster, the bitter and pungent notes were appreciated, whereas sweet taste and crisp mouthfeel were less definite. There was a substantial decline in preference for increased sweetness in 2000, the year marked by generally higher levels of sweetness. The optimal sweetness intensity for this consumer group seemed to have been surpassed.

The separation of the attributes which increased acceptability from those which decreased it was possible not only when based on the differences between the broccoli and cauliflower groups in one data set, but also, when only broccoli samples were used. Fig. 9 shows the distribution of the correlation coefficients for the relationship between the attribute intensities and consumer acceptance as in Fig. 8, but in this case it was calculated only for the broccoli samples.

In 1998 and 1999 bitter and pungent notes within the broccoli sample group were also negatively correlated with acceptance, whereas cauliflower-flavour, sweetness and crisp mouthfeel gave positive coefficients. In 2000, the range of

Table 7 - Correlation coefficients for the relationship of attribute intensity and consumer acceptance. Percentage of consumers assigned to the cluster. Broccoli and cauliflower samples included.

		Cluster 1		Cluster 2		Cluster 3	
Taste bitter	1998	-0.50	43%	-0.23	36%	+0.50	21%
	1999	-0.72	55%	-0.29	26%	+0.40	19%
	2000	-0.79	35%	+0.49	38%	+0.31	27%
Flavour pungent	1998	-0.42	43%	-0.28	36%	+0,51	21%
	1999	-0.58	55%	-0.46	26%	+0.41	19%
	2000	-0.39	35%	-0.53	38%	+0.84	27%
Taste sweet	1998	-0.22	43%	+0.64	36%	+0.09	21%
	1999	+0.01	55%	+0.56	26%	-0.35	19%
	2000	+0.39	35%	+0.53	38%	-0.84	27%
Mouthfeel crisp	1998	+0.53	43%	-0.01	36%	-0.21	21%
	1999	+0.35	55%	-0.32	26%	+0.19	19%
	2000	+0.75	35%	-0.61	38%	-0.17	27%

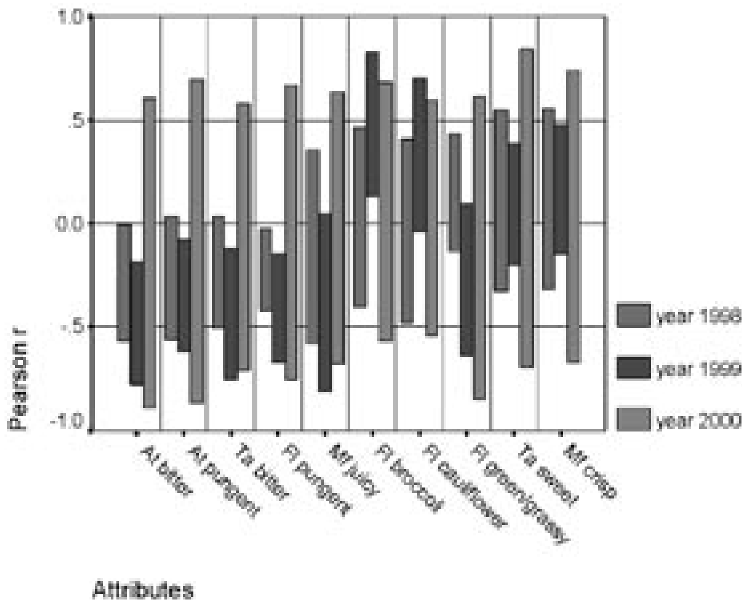

Fig. 9 - Distribution of the correlation coefficients for the relation of product attributes and individual consumer acceptability. Broccoli samples only. At=aftertaste, Fl=flavour, Mf=mouthfeel, Ta=taste.

intensities of the tested broccoli samples was very narrow, which probably led to a larger distribution of the preference correlation for greater or lesser intensities of the attributes. The separation into consumer clusters (Table 8) gave almost the same results as the calculation, which included the cauliflower samples (Table 7). Within the broccoli samples alone, a large group of consumers (cluster 1) rejected increased bitter and pungent notes, and preferred crisp mouthfeel or sweetness (cluster 2). In cluster 3 the positive correlation be-

Table 8 - Correlation coefficients for the relationship of attribute intensity and consumer acceptance. Percentage of consumers assigned to the cluster. Only broccoli samples included.

		Cluster 1		Cluster 2		Cluster 3	
Taste bitter	1998	-0.57	45%	-0.30	27%	+0.10	27%
	1999	-0.58	66%	-0.22	27%	+0.26	7%
	2000	-0.19	39%	-0.60	41%	+0.46	20%
Flavour pungent	1998	-0.47	45%	-0.35	27%	-0,04	27%
	1999	-0.59	66%	-0.26	27%	+0.28	7%
	2000	-0.27	39%	-0.60	41%	+0.45	20%
Taste sweet	1998	+0.32	45%	+0.63	27%	+0.21	27%
	1999	+0.11	66%	+0.22	27%	-0.24	7%
	2000	+0.24	39%	+0.61	41%	-0.45	20%
Mouthfeel crisp	1998	+0.56	45%	+0.50	27%	-0.07	27%
	1999	+0.39	66%	+0.30	27%	-0.32	7%
	2000	+0.56	39%	-0.10	41%	-0.07	20%

tween bitter and pungent notes and acceptability remained, but with lower average coefficients.

CONCLUSION

Three years of experiments showed that it was possible to perceive and assign many sensory attributes from which a wide variety of broccoli and cauliflower samples could be distinguished. Besides the differences among the samples, large variations were also attributable to the climatic conditions during cultivation in each of the three years. The attributes, which were most suitable for differentiating the sample and/or the year of cultivation were: bitter and sweet taste, broccoli-like and cauliflower-like flavour, green/grassy and pungent flavour, juicy and crisp mouthfeel and bitter and pungent aftertaste. PCA analysis disclosed the main source of the differences among the samples. More than half (56%) of the variation in the data could be assigned to differences in the attributes of bitter, pungent and green/grassy notes on the one hand, and sweetness, cauliflower-flavour and crispness on the other hand. Sensory differentiation was very noticeable between the traditional white "Marine" cauliflower and Chinese broccoli. Some cauliflower and broccoli samples were able to be separated, while others overlapped.

A synopsis of the sensory attributes and sugar content in the samples showed, that sweetness cannot be predicted from the sugar content alone. Sweetness was much more (negatively) related to the total glucosinolate content, which, in turn coincided closely with bitter and pungent notes.

Increasing intensity of these notes led, on average, to reduced acceptability of both cauliflower and broccoli samples. Cluster analysis identified a small consumer segment that appreciated for bitter and pungent notes; this segment could be considered to be a niche segment. The majority of consumers preferred samples with lower intensities of bitter and pungent notes; these were assigned to samples with a glucosinolate content of 30 to 35 mg/100 g FM or less. Below this value, there was no correlation between glucosinolate content and bitter or pungent notes.

To avoid losses of acceptability due to reduced sweetness and increased incidence of bitter and pungent notes, care has to be taken when selecting cultivars. Cultivars, which do not tend to become bitter or pungent especially at low growing temperatures should be chosen.

This sensory consumer study can be considered a starting point for guiding the "design" of altered products which will appeal to target consumer populations and for integrating hedonic and health benefits of *Brassica* vegetables.

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PAPER

ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS IN THE EDIBLE PARTS OF EARLY AND LATE ITALIAN ARTICHOKE (*CYNARA SCOLYMUS* L.) VARIETIES

ATTIVITÀ ANTIOSSIDANTE E COMPOSTI FENOLICI NELLE PARTI EDULI DI VARIETÀ ITALIANE PRECOCI E TARDIVE DI CARCIOFO (*CYNARA SCOLYMUS* L.)

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ABSTRACT

The content of total phenolic compounds (TPC) and chlorogenic acid (CA) and the antioxidant activity in the methanolic extracts of edible parts of three early and two late Italian artichoke (*Cynara scolymus* L.) varieties were determined and compared. TPC content was determined by the Folin-Ciocalteu reagent and the antioxidant activity by the DPPH method. CA quantification was carried out by HPLC and GC/MS using CA methyl ester as internal standard. Results showed that

RIASSUNTO

Estratti metanolici delle parti eduli dei capolini centrali di tre varietà precoci e due varietà tardive di carciofo (*Cynara scolymus* L.) sono stati analizzati e confrontati per il contenuto di polifenoli totali (TPC), di acido clorogenico (CA) e per l'attività antiossidante. Il TPC è stato determinato mediante il reagente di Folin-Ciocalteu, l'attività antiossidante utilizzando il radicale DPPH. Il CA è stato quantificato mediante HPLC e GC/MS, usando il metilestere del CA come standard interno. I risultati evi-

- Key words: antioxidant activity, artichoke heads, chlorogenic acid, DPPH, GC/MS, TPC -

artichoke varieties differ considerably in their TPC (7.31-13.05 mg/g fresh weight) and CA (1.36-2.46 mg/g fresh weight) content, as well as in their antioxidant activity. No significant correlation was observed between TPC content and DPPH scavenging activity. Boiling the inner bracts of the heads led to a 46% loss of CA.

denziano che le singole varietà di carciofo differiscono considerevolmente sia per il contenuto di TPC (7,31-13,05 mg/g peso fresco) e di CA (1,36-2,46 mg/g peso fresco), sia per l'attività antiossidante. Non è stata trovata una correlazione significativa fra contenuto di TPC e attività antiossidante. La bollitura delle brattee interne dei capolini, una comune procedura di cottura del carciofo, ha comportato una perdita del 46% del contenuto di acido clorogenico.

INTRODUCTION

Artichoke (*Cynara scolymus* L.), a typical Mediterranean crop belonging to the Asteraceae, is cultivated worldwide and, according to FAO (1999), Italy is the main artichoke producer in the world. Artichoke heads have long been a part of the traditional Mediterranean diet and many cultivars or ecotypes are grown in Italy and other Mediterranean countries.

Artichoke is known to be a good source of polyphenols. There is growing evidence that many phenolic metabolites may exert beneficial effects on human health, at least partly attributable to their antioxidant and free radical scavenging activity (CASTELLUCCIO *et al.*, 1995; PARR and BOLWELL, 2000).

For a long time, artichoke leaves have been used in herbal medicine for their choleretic and diuretic effects. Leaf extracts of artichoke have been shown to have marked antioxidative, hepatoprotective, choleretic and hypocholesterolemic activities in various *in vitro* and *in vivo* test systems (KIRCHHOFF *et al.*, 1994; GEBHARDT and FAUSEL, 1997; KRAFT, 1997; BROWN and RICE-EVANS, 1998) and are widely used for therapeutic purposes.

The bioactive components of artichoke extracts are a complex mixture of several polyphenols (i.e. caffeic acid, chlorogenic acids, luteolin, scolymoside and cynaroside). The quantitatively predominant compound is chlorogenic acid (3-*O*-caffeoyl-D-quinic acid, CA), a hydroxycinnamate that is widespread throughout the plant kingdom (CLIFFORD, 1999; KROON and WILLIAMSON, 1999). Chlorogenic acid shows strong antioxidant activity and may inhibit the oxidation of low density lipoproteins, thus acting as a naturally occurring dietary substance that may reduce and/or prevent atherogenesis, coronary heart disease and human carcinogenesis (LARANJINHA *et al.*, 1994; CHEN and HO, 1997; KONO *et al.*, 1997; 1998; LARSON, 1998; MEYER *et al.*, 1998; LO and CHUNG, 1999).

Despite the vast literature that refers to other plant sources, and the extensive studies on artichoke leaves (ADZET and PUIGMACIA, 1985; FRITSCHE *et al.*, 2002), few data are available about the total phenolic compound and chlorogenic acid content and the antioxidant activity of the edible parts (inner bracts or "hearts") of artichoke heads (WANG *et al.*, 2003; ALAMANNI and COSSU, 2003; JIMÉNEZ-ESCRIG *et al.*, 2003). Furthermore very little consideration has been given to the content of polyphenolic compounds in heads at different develop-

mental stages or to the effect of common cooking procedures (i.e. boiling).

Different procedures have been used to determine the orthodiphenolic content of artichoke extracts (NICHIFORESCU, 1970; LATTANZIO and VAN SUMERE, 1987; HAMMOUDA *et al.*, 1993; WANG *et al.*, 2003). The separation of single phenolic components (i.e. CA) from complex mixtures such as plant extracts is a difficult, time-consuming task. Quantitative data on the content of the polyphenol composition of edible artichoke parts has often been obtained using unreliable methodologies that do not consider the loss that occurs during extract purification. In addition, CA is rather unstable and easily undergoes isomerization by intramolecular transesterification and photo-isomerization from the natural *trans*- isomer to the *cis*- isomer when exposed, even briefly, to daylight (MOLLER and HERRMANN, 1982; IGLESIAS *et al.*, 1985; FUCHS and SPITELLER, 1996) during sample preparation and storage; this frequently causes extraction artefacts, making it even more difficult to quantify the analyte.

Little information is currently available on the total phenolic content, the concentrations of the single polyphenolic compounds, or the antioxidant properties of different artichoke varieties. However, quantitative data on the polyphenol content and antioxidant properties of the edible parts of different artichoke varieties are needed to evaluate the nutritional quality of the fresh product (TOMÁS-BARBERÁN and ESPÍN, 2001). This information will also allow artichoke varieties to be selected on the basis of their phenolic profile, which is of interest because most modern varieties have been developed with more emphasis on agronomic, rather than nutritional performance. Artichoke extracts could also be used as sources of natural antioxidant compounds, mainly polyphenols, which, in some cases, have activities comparable to those of synthetic antioxidants.

The aim of the present work was to determine: 1) the TPC content by the Folin-Ciocalteu reagent; 2) the free radical scavenging activity of raw extracts by DPPH (2,2-diphenyl-1 picrylhydrazyl) test and 3) the CA content at the harvest stage, and its variation during the development of the main head, by means of gas chromatography/mass spectrometry (GC/MS) using CA methyl ester as internal standard. The effect of a thermal treatment, commonly used in home cooking, on the CA content of edible parts was also assessed.

MATERIALS AND METHODS

Chemicals and standards

3-*O*-caffeoyl-D-quinic acid (chlorogenic acid, CA) and 2,2-diphenyl-1 picrylhydrazyl (DPPH), Folin-Ciocalteu reagent and 1 mL semi-micro polystyrene cuvets were purchased from Sigma-Aldrich S.r.l. (Milan, Italy). N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) was obtained from Pierce Biotechnology, Inc.(Rockford, Illinois, USA). Methanol (MeOH) was purchased from Scharlau Chemie (Barcelona, Spain); ethyl acetate (EtOAc) and sodium carbonate (Na_2CO_3) were obtained from J.T. Baker (Deventer, Holland). All the solvents used were double-distilled and HPLC grade.

Plant material

Cynara scolymus L. plants of the early varieties "Violetto di Provenza" and "Locale di Mola" were grown in the fields of the Istituto Sperimentale di Orticoltura (Pontecagnano, Salerno, Italy); plants of the early variety "Violetto di Sicilia" were grown in a field in Siracusa, Sicilia, Italy. The plants of the late variety "Grato 1" were grown by A.R.S.I.A.L. (Azienda Regionale per lo Sviluppo e l'Innovazione in Agricoltura del Lazio), in Cerveteri,

Rome, Italy. The plants of the late variety "Terom" were grown in the experimental fields of the Dipartimento di Biologia delle Piante Agrarie located in S. Piero a Grado, Pisa, Italy. For each variety, the main heads were harvested at the mature stage from plots of at least 30 plants. In order to determine the changes in CA content during head development, the main heads were also collected 26 and 14 days before the mature stage from the "Terom" variety and 15 and 7 days before the mature stage from the "Grato 1" variety. Secondary heads were harvested from "Terom" plants 10 days after the mature stage of the main heads and from "Grato 1" plants 14 days after the mature stage of the main heads. After harvesting, the heads were stored at -80°C until extraction. In order to quantify the effect of boiling on the CA content, five secondary heads of the variety "Grato 1" were each cut in half: five halves were boiled for 20 min and then analysed, while the remaining halves were directly analysed. The percentage of dry matter of the edible parts of the heads was determined after reducing the fresh heads to dryness in an oven at 70°C for 7 days; the percentage ranged from 6.9 to 7.2 for all the cvs analysed.

Determination of total phenolic compounds (TPC)

The TPC content was measured according to the Folin-Ciocalteu method previously described by SHADID and NACZK (1995). For each variety, 5 g (fresh weight, f.w.) of edible parts (1 g from each of 5 artichoke heads collected from 5 different plants) were extracted with 80% MeOH. The edible parts were prepared by removing the outer fibrous bracts and the stem cortex. The samples were frozen with liquid nitrogen, homogenised and extracted (3 times) with 80% MeOH for 12 h at 4°C. In preliminary experiments it was found that further residue extractions did not increase the extraction efficacy. The methanolic extracts were pooled and centrifuged at 4,000 g for 20 min. Raw extracts were stored in the dark at –20° C until analysis. Different dilutions of the 80% MeOH extracts (0.25 mL) were mixed with 0.25 mL of Folin-Ciocalteu reagent (diluted with distilled H_2O 1:1 v/v), 0.5 mL of a saturated Na_2CO_3 solution and 4 mL of H_2O. The reaction mixtures were left at room temperature for 30 min, and then centrifuged at 4,000 g for 15 min. Supernatant absorbance (Abs) was measured at 725 nm using a Hitachi U-3200 spectrophotometer (Hitachi Ltd, Tokyo, Japan). Each artichoke extract was analysed three times, and each single analysis was performed in triplicate. Extract dilutions corresponded to 10, 5, 2.5, 1.25, 0.625, 0.250, 0.0625 mg f.w.. A calibration curve was obtained by mixing different quantities of CA standard (0, 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 75 and 100 µg) with 0.25 mL of Folin-Ciocalteu reagent. TPC content is expressed as mg CA equivalents/g f.w. (± SD, *n*=3).

Determination of antioxidant activity

The antioxidant activity of the extracts was measured by means of a free radical method using the DPPH test (BRAND-WILLIAMS *et al.*, 1995). In this spectrophotometric assay, the antioxidant activity is evaluated by measuring the reduction of the stable radical DPPH by antioxidants ($DPPH^{\bullet} + A \rightarrow DPPH{-}H + A^{\bullet}$) or by radical species ($DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH{-}R$) which results in loss of its absorbance at 515 nm. For DPPH analysis, a 153 µM stock solution of DPPH in 80% MeOH was used. Analyses were made using a Hitachi U-3200 spectrophotometer. A standard curve for DPPH was obtained measuring the absorbance at 515 nm (Abs_{515}) of nine dilutions of the free radical (153, 125, 100, 80, 75, 60, 50, 25, 10 µM). Analyses were performed by adding 950 µL of a 153 µM DPPH solution to 50 µL of diluted raw extract or methanolic CA standard solution. Extract dilutions were prepared by

mixing 37.50, 31.75, 25.00, 18.75, 12.50, 6.25 µL of each extract with H_2O up to a final volume of 50 µL. To assess the radical scavenging activity of the CA standard, the following amounts were used: 0.5, 1, 2, 5, 10, 20, 30 µg. The Abs_{515} of the samples was read against a blank of 80% MeOH without DPPH. Raw extracts of each variety, prepared as previously described for TPC analysis, were analysed three times (±SD, *n*=3). The Abs_{515} of the samples was immediately read after mixing DPPH with the raw extract (0 min = start reaction), then after 30, 60, 120, 180, 240, 360, 480 min and 24 h, or until the reaction reached a plateau. After each reading, cuvets were stored in the dark at room temperature. For each varietal extract, different molar ratios (moles of TPC, expressed as CA equivalents, per mole of DPPH) were tested. The exact initial DPPH concentration (C_{0DPPH}) of each sample was calculated using the following linear regression equation ($C_{0DPPH} = 7.99\times10^{-5} x Abs_{515} + 2.06\times10^{-7}$) as previously reported by BONDET *et al.* (1997). The percentage of remaining DPPH was determined for each time interval and the reaction kinetics for each molar ratio were plotted (% of remaining DPPH against time). Based on these reaction kinetics, the percentage of the remaining DPPH at the steady state was calculated and plotted against the molar ratios. The EC_{50} (molar ratio corresponding to 50% of the remaining DPPH) was determined for each artichoke extract tested. The antiradical power (ARP) as defined by BRAND-WILLIAMS *et al.* (1995) was then determined.

Quantification of CA

Four aliquots of the raw methanolic extract used for the TPC analysis (each corresponding to 0.05 g f.w.) were analysed separately for the CA content. One hundred µg of CA methyl ester was added to each aliquot as internal standard, reduced to dryness under vacuum and re-dissolved in 10 mL of distilled H_2O. The samples were then partitioned five times against EtOAc (v/v=1:1) after adjusting the pH to 2.5 with HCl. The organic phases were stored overnight in complete darkness at -20°C; the frozen H_2O was separated and the EtOAc was removed by evaporation. The dry extract was re-suspended in 500 µL of 10% MeOH +0.1% CH_3COOH for RP-HPLC purification. During these procedures the extracts were kept away from direct light sources, in order to minimise photoisomerization of the analyte.

Preparation of internal standard

CA methyl ester was obtained by methylation with diazomethane from a methanolic standard solution of CA (SCHLENK and GELLERMAN, 1960).

RP-HPLC purification

A Kontron 422 reversed-phase high pressure liquid chromatograph (Kontron Instruments SpA, Milan, Italy), equipped with a spectromonitor LDC 3200 variable wavelength UV detector, was used. The volume injected was 500 µL. Solvents: (A) H_2O + 0.1% CH_3COOH; (B) MeOH.

Preparative RP-HPLC

Column: Lichrosorb RP 18, 10 µm, 150x10 mm i.d., detection wavelength = 325 nm; flow rate = 4 mL min^{-1}. Elution conditions: isocratic 10% B in A for 11 min; linear gradient from 10 to 37% B in A for 1 min; isocratic 37% B in A for 10 min; linear gradient from 37 to 100% B in A for 2 min. CA elution time (t_E) = 14 min; CA methyl ester t_E = 16 min.

Analytical RP-HPLC and derivatization

Column: Hypersil ODS, 5 µm, 250x4.6 mm i.d.; detection wavelength = 325 nm;

flow rate = 1.1 mL min^{-1}. Elution conditions: isocratic 20% B in A for 8 min; linear gradient from 20 to 37% of B in A for 1 min; isocratic 37% B in A for 11 min; linear gradient from 37 to 100% B in A for 2 min; CA t_E = 13 min; CA methyl ester t_E = 17 min.

After HPLC purification, the eluates containing the CA fraction were transferred into microvials, dried and silylated with 100 µL BSTFA + 1% TMCS at 80°C for 1 h.

GC/MS analysis

GC/MS was performed on a VG Trio 2000 mass spectrometer coupled with a Hewlett-Packard 5890 series II gas chromatograph (VG Biotech, Altrincham, Cheshire, UK) equipped with a crosslinked Ultra 1 capillary column (25 m x 0.32 mm i.d.; 0.52 µm film thickness). The GC temperature conditions were: 200°C for 3 min; from 200° to 270°C at 30°C min^{-1}; from 270° to 310°C at 4°C min^{-1}; 310°C for 5 min. TMS-CA (trimethylsilylated chlorogenic acid) t_R = 8.30 min; TMS-CA methyl ester t_R = 8.45 min. The carrier gas was helium at a flow rate of 25 mL min^{-1}. Electron impact (EI^+) mass spectra were recorded at an ionisation energy of 70 eV. Data acquisition was from 70 to 800 amu at 600 amu s^{-1} in full scan mode. Trimethylsilylation of CA allowed a rapid identification of the analyte by means of characteristic key ions at 255, 345, 307 and 786 m/z (FUCHS and SPITELLER, 1996; BOMBARDELLI *et al.*, 1977).

CA quantification by GC-MS

Quantitative analyses were performed by comparing the area of ions 728 and 786 (m/z 728 = M^+ of TMS-CA methyl ester; m/z 786 = M^+ of TMS-CA) in the mass spectra of the samples. A calibration curve was prepared by mixing a constant amount of the internal standard (CA methyl ester, 20 ng) with different amounts of CA (0, 20, 50, 100, 200 ng). A graph was constructed by plotting 786:728 peak area ratios against the amount of CA. The calibration curve showed a strong linear regression with a correlation coefficient of r^2 = 0.994. The detection limit of CA in these GC-MS conditions was 1 ng.

The following formula was used for CA quantification:

$$\text{Endogenous amount of CA } (\mu g) = \frac{A}{B} \times 100$$

Where A is the abundance of ion at m/z 786 and B is the abundance of ion at m/z 728 and 100 (µg) is the amount of internal standard added to the samples. Final data were the means of four GC/MS analyses (±SD, *n*=4).

RESULTS AND DISCUSSION

TPC of methanolic artichoke extracts

The TPC content of main heads (inner bracts) of artichoke at the harvest stage is shown in Table 1. TPC ranged from 13.05 mg CA equivalents/g f.w. in "Grato 1" to 7.31 mg CA equivalents/g f.w. in "Terom". Expressed on a dry weight ba-

Table 1 - Total phenolic compound (TPC) content in the methanolic extracts of artichoke heads at the mature stage, expressed in mg CA equivalents/g f.w., (±SD, *n*=3).

Variety	TPC
Violetto di Sicilia°	11.72±1.46
Violetto di Provenza°	12.32±1.10
Locale di Mola°	12.72±2.46
Terom *	7.31±0.36
Grato 1*	13.05±1.58

° Early varieties
* Late varieties

sis, the TPC values ranged from 10.43 to 18.63%. WANG *et al.* (2003) reported that the total phenols in young and mature heads of three American artichoke varieties ranged from 1.6 to 3.1% on a dry weight basis, which are much lower than our values. The difference may be due to several factors including type of material analysed (inner edible bracts or whole heads), developmental stage, varietal differences and growth conditions. Significant differences in radical scavenging activity and TPC were reported from bran extracts prepared from the same wheat variety grown in different locations (YU *et al.*, 2002), indicating that environmental conditions may alter the antioxidant properties of a particular variety. Similar conclusions about changes in the antioxidant properties in strawberry due to differences in growing temperatures were reported by WANG *et al.* (2001). High TPC content in the edible parts of artichoke varieties grown in the Mediterranean area has also been reported by DELLA GATTA and PATRUNO (1976) (8.71 mg/g f.w.), AUBERT and AMIOT (1986) (9 mg/g f.w., the sum of the single components), LATTANZIO and VAN SUMERE (1987) (3-6% of dry weight) and ALAMANNI and COSSU (2003) (var. spinoso sardo, 5.89 mg/g f.w.). A high total phenolic content may therefore be a property of edible artichoke heads from plants grown in the Mediterranean area, as a consequence of different genotypic backgrounds and/or growing conditions.

DPPH scavenging activity of methanolic artichoke extracts

The extracts from the different artichoke varieties had similar kinetic reaction curves against the DPPH radical as shown for "Grato 1", "Terom" and "Violetto di Sicilia" in Fig. 1. Both dose- and time-dependent effects were observed. Higher concentrations of artichoke extracts were more effective in scavenging the free radical in the system. Table 2 shows the antiradical power values for the different artichoke varieties, obtained by using the DPPH test. The early variety "Violetto di Sicilia" had the highest ARP value (10.85), followed by the early variety "Locale di Mola" and the late variety "Grato 1" (8.73 and 8.67, respectively), while the late variety "Terom" had the lowest ARP value (4.85).

All the extracts, with the exception of the Terom variety, when compared in the same system, showed better antiradical

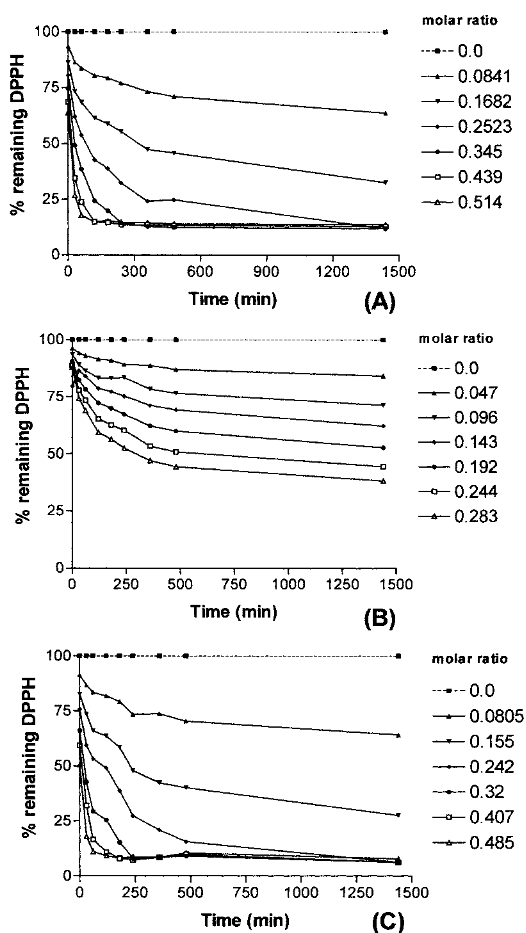

Fig. 1 - Percentage of remaining DPPH vs time for: (A) var. "Grato 1"; (B) var. "Terom"; (C) var. "Violetto di Sicilia". Molar ratio: moles of TPC, expressed as CA equivalents per mole of DPPH.

Table 2 - Antiradical power (ARP) of methanolic extracts of artichoke heads at the mature stage (±SD, *n*=3).

Variety	ARP
Violetto di Sicilia°	10.85±1.28
Violetto di Provenza°	6.51±1.50
Locale di Mola°	8.73±1.56
Terom*	4.85±0.01
Grato 1*	8.67±0.13

° Early varieties
* Late varieties

power than pure CA, which had an ARP value of 6.25.

No apparent differences were observed between the early and late varieties, with respect to the radical scavenging activity of edible head extracts. Likewise no significant correlation was observed between radical scavenging activity for DPPH radicals and the TPC content of the edible parts of artichoke heads (r^2=0.26). This observation is not in agreement with that reported by WANG *et al.* (2003). They found that the scavenging activity for the DPPH free radical of different artichoke samples was highly correlated to the total phenol content. ZIELINSKI and KOZLOWSKA also found (2000) a high correlation coefficient between total antiradical activity and total phenolic compounds in wheat extracted with 80% methanol. However, no linear relationship was observed between antiradical activity of water extracts and their phenolic content. YU *et al.* (2002) reported different capabilities of wheat varieties to quench DPPH and ABTS radicals, with no correlation between total antiradical activity and total phenolics extracted with absolute ethanol. These contrasting observations may be explained by genotypic, as well as environmentally-induced differences in the composition of phenolic compounds, and/or by other components that in addition to the phenolic compounds may react directly with radicals. This may well be the case for different artichoke varieties. Vitamin C content in the edible parts of artichoke extracts can be relatively high (GIL-IZQUIERDO *et al.*, 2001) and may have significant antioxidant activity in the DPPH assay (SANCHEZ-MORENO *et al.*, 2003). Artichoke extracts are rich in several polyphenolic compounds: i.e. anthocyanidins, anthocyanins, caffeic acid, mono- and di-caffeoylquinic acids, flavonoids and conjugated forms, such as luteolin and cynaroside. The various polyphenolic components possess different antioxidant and radical-scavenging activities (GEBHARDT and FAUSEL, 1997; CHEN and HO, 1997; HAMMOUDA *et al.*, 1993; WANG *et al.*, 2003) and thus contribute differently to the overall antioxidant activity of the extract. Moreover, the single phenolics may exhibit antagonistic/synergistic effects among themselves or other compounds in the extracts, resulting in a total antioxidant activity of the raw extracts that is greater than the sum of the individual compounds (RICE-EVANS *et al.*, 1996).

CA content in the main heads of early and late artichoke varieties

Main heads (internal bracts or "hearts"), collected at harvest time from all of the varieties were analysed to determine the CA content. As reported in Table 3, the CA content varied great-

Table 3 - Chlorogenic acid (CA) content in the inner bracts of main artichoke heads at the mature stage, expressed in mg/g f.w. (±SD, *n*=4).

Variety	CA
Violetto di Sicilia °	1.53±0.02
Violetto di Provenza °	2.15±0.19
Locale di Mola °	2.18±0.09
Terom *	1.36±0.59
Grato 1*	2.46±0.28

° Early varieties
* Late varieties

ly, ranging from 1.36 mg/g f.w. in "Terom" to 2.46 mg/g f.w. in "Grato 1" (corresponding to 2 and 3.5% of total dry weight, respectively). No correlation was found between radical scavenging activity for DPPH radicals and CA content of the edible parts of artichoke heads (r^2 = 0.040).

Most data on the CA content of artichoke are related to non-food sources (ADZET and PUIGMACIA, 1985; HINOU *et al.*, 1989; BEN-HOD *et al.*, 1992, FRITSCHE *et al.*, 2002). However, there are a few reports on the polyphenolic components in the edible portion of artichoke heads (DELLA GATTA and PATRUNO, 1976; LATTANZIO, 1981; LATTANZIO and MORONE, 1979; ALAMANNI and COSSU, 2003; WANG *et al.*, 2003). Even though different varieties of artichokes have occasionally been compared (WANG *et al.* 2003), it is rare that the physiological stage of head development has been considered (LATTANZIO and MORONE, 1979). However, the CA content of the edible parts of the main heads in different artichoke varieties at harvest is of great importance in order to evaluate the nutritional quality of the fresh product. The few reports available on the CA content in artichoke heads show a wide range of values (1-5 mg/g f.w.). Marked differences have even been reported for the same variety; i.e. for "Violetto di Provenza", LATTANZIO and MORONE (1979), reported a CA content of 4.73 mg/g f.w., while AUBERT and AMIOT (1986) reported a value of 1.5 mg/g f.w.. This variability may be attributed, at least partially, to the different analytical methods (TLC, PC, UV spectrophotometry, gel-chromatography and HPLC) used for the quantitative determination of CA in artichoke extracts.

To our knowledge, none of the previous quantitative studies were carried out using a suitable internal standard in order to calculate losses during the purification procedure. This, along with the limited selectivity of the frequently used analytical techniques, may have underestimated/overestimated the real content of the single analytes, which would, in turn hamper a reliable comparison among different artichoke varieties. Our results indicate a significant variability among different artichoke varieties, even when the CA content was determined by rigorous quantitative methods. The level of other phenolic components in artichoke heads may also vary, highlighting the possibility of selecting artichoke varieties with desired antioxidant properties. As for TPC content, the endogenous concentration of CA (2.0-3.5% dry weight), for all the Italian varieties analysed, was higher than that reported by WANG *et al.* (2003) for the American varieties "Imperial Star" and "Green Globe" (0.1-0.5% dry weight). A high CA content (2.47 mg/g fresh weight) was also reported by ALAMANNI and COSSU (2003) for the var. *spinoso sardo.*

Dynamics of CA content during head development

Variations in the CA content during head development was determined for the two late varieties, "Grato 1" and "Terom" (Fig 2A). The results show that the CA content in the inner bracts of the main heads decreased constantly during head development until the mature stage. In "Grato 1" the CA concentration decreased 37.5% (from 3.94 mg/g f.w. at 26 days before the mature stage to 2.46 mg/g f.w. at the mature stage). A similar tendency was observed in the inner bracts of the main heads of "Terom", which showed a 56% decrease in the CA concentration (from 3.11 mg/g f.w. 15 days before the mature stage, to 1.36 mg/g f.w. at the mature stage). The CA content in the inner bracts of the secondary heads, sampled after the harvest of the main heads (10 days and 14 days for "Terom" and "Grato 1" respectively), was higher (2.23 mg/g f.w. in "Terom"

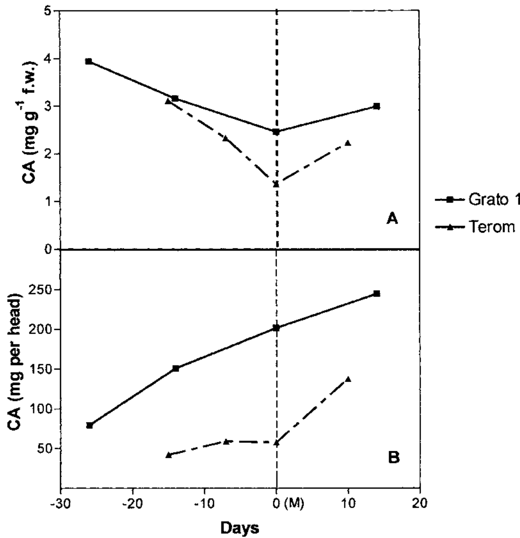

Fig. 2 - Chlorogenic acid (CA) content in inner bracts during the development of main heads and, after the mature stage, in secondary heads. A: CA concentration in mg/g f.w. (±SD, *n*=4); B: CA content per head. M = mature stage.

and 2.99 mg/g f.w. in "Grato 1") than the concentration in the main heads at the harvest stage (1.36 mg/g f.w. in "Terom" and 2.46 mg/g f.w. in "Grato 1"). When the amount of CA was calculated on a per-head basis, the CA content increased steadily with head growth (Fig. 2B).

Effect of cooking on the CA content

Home cooking and industrial processing of fresh vegetables may cause major changes in the polyphenolic content depending on the nature and duration of the treatment (DAO and FRIEDMAN, 1992; GAZZANI *et al.*, 1998). The variations in the CA content in the edible parts (inner bracts) of artichoke heads were determined after boiling in water for 20 min, a procedure that is commonly used in home cooking. The GC/MS quantitative determination of the CA content in the inner bracts before boiling showed a value of 2.99 mg/g f.w., while after boiling the CA concentration decreased to 1.39 mg/g f.w (54% of the initial value). The observed CA loss (about 46%) is on the same order as that reported for CA content in cooked potatoes (DAO and FRIEDMAN, 1992).

CONCLUSIONS

This study shows that artichoke varieties differ significantly in their free radical scavenging properties and TPC. The lack of a significant correlation between free radical scavenging properties and TPC may be due to a different polyphenol composition as well as the probable presence of other components (like vitamin C) in the extracts of the different varieties. Since past data on the content of individual polyphenols in artichoke heads were often obtained using inaccurate quantitative methodologies, we analysed the content of the main phenolic compound (CA) in the different varieties with a reliable methodology based on RP-HPLC and GC/MS and used CA methyl ester as internal standard. Our results indicate that additional studies are needed to investigate the phenolic components that contribute to the total antioxidant activity of different artichoke varieties, particularly considering the many cultivars or ecotypes grown in Italy and other Mediterranean countries. Information from such studies could then be used to produce selected artichoke varieties that have a desired phenolic composition with high antioxidant properties.

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A TENTATIVE MATHEMATICAL MODEL TO DESCRIBE THE EVOLUTION OF PHENOLIC COMPOUNDS DURING THE MACERATION OF SANGIOVESE AND MERLOT GRAPES

UN MODELLO MATEMATICO PER DESCRIVERE L'EVOLUZIONE CINETICA DELL'ESTRAZIONE DELLE DIVERSE FRAZIONI POLIFENOLICHE DA UVE SANGIOVESE E MERLOT NEL CORSO DELLA MACERAZIONE

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ABSTRACT

The maceration of the solid parts (dregs) of grapes is a fundamental step of wine-making not only for the production of high quality red wines but also of structured white wines characterized by a high content of aromatic compounds. A mathematical model able to describe the time evolution of the phenolic fractions during grape maceration is reported and discussed. While the accumulation of total phenols and catechins in the must/wine during

RIASSUNTO

La macerazione delle parti solide dell'uva rappresenta uno stadio fondamentale nella preparazione non solo dei grandi vini rossi ma anche dei bianchi strutturati e ricchi in componenti aromatici. Viene introdotto ed analizzato un modello matematico in grado di descrivere l'evoluzione nel tempo del processo estrattivo a carico della componente polifenolica delle uve sottoposte a macerazione. Mentre per descrivere l'evoluzione nel tempo della diffusione nel mo-

- Key words: anthocyanin oxidation, extraction selectivity, extraction yield, mathematical model, phenol extraction, time evolution, wine-making -

grape maceration can be described by the mass transfer equation introduced by Fick to evaluate the diffusion occurring between two heterogeneous phases, a more complex mathematical approach is introduced to determine the time evolution of anthocyanins. The extraction rate of different phenolic compounds, does not seem to vary significantly with the kind of fermentor or the wine-making technology used, but both the yield and chemical composition of the extracts appear to be significantly affected by extraction time.

sto/vino dei polifenoli totali e delle catechine, è stata impiegata un espressione ottenuta elaborando l'equazione di Fick, per analizzare quella degli antociani si è dovuto utilizzare un sistema matematico più complesso.

Se il tipo di fermentatore utilizzato o la tecnica di omogeneizzazione impiegata non sembrerebbero in grado di condizionare significativamente la cinetica estrattiva, il tempo di macerazione agirebbe non solo modificando sensibilmente la resa ma anche e soprattutto la selettività del processo e quindi la composizione degli estratti ottenuti.

INTRODUCTION

The maceration of the solid parts of grapes is a fundamental step of wine-making not only in the production of high quality red wines (GLORIES, 1978) but, also of white wines. In fact, the extraction of phenolic and aromatic compounds from the grape occurs during maceration, and the subsequent wine-making steps are significantly influenced by the length and intensity of this phase (RIBEREAU GAYON *et al.*, 1970). When the phenolic compounds are extracted together with the other substances, a large amount of the tannic fraction is dissolved in the must (OH *et al.*, 1980). In such a case, the tannins coming from the grape skins, as well as those from the seeds, are transferred into the liquid phase. The strong astringent taste of many wines, as well as the possibility of prolonging the wine life, are attributable mainly to these phenolic compounds. The production of high quality wines, to be stored for a long time in wooden tanks (barriques and barrels), also involves the interaction of tannins with anthocyanins to increase and stabilise the colour (co-pigmentation). The colour, clarity and taste of these wines increases with wine ageing as the aromatic profile becomes more complex and pleasing.

Temperature, the addition of SO_2 and maceration time appear to be the working parameters that affect the extraction yield of phenolic compounds (VIVAS *et al.*, 1992). The extraction of phenolic compounds has to be carefully regulated with respect to the type of grapes, their state of health and the desired end wine to be produced. If the wine is to have a limited commercial life, a short maceration of solid parts should be carried out, while, for a more complex wine destined to be maintained for a long time, a longer maceration and more extensive extraction should be used. In this latter case, particular working conditions (pre-fermentative cold maceration, extraction at high temperatures with a high SO_2 level, etc.) or the addition of oenological coadjuvants (pectolitic enzymes, tannins, etc.) could help increase the content and stability of the phenols extracted in the liquid phase (must/wine). The stability of phenols, particularly anthocyanins, is strongly related to the concentration of dissolved oxygen in the must. As extensively reported in the literature, the time evolution of the anthocyanin concentration in must during grape macer-

ation, has a maximum due to the effect of at least two opposing processes: extraction from grape skin and successive transformations.

A mathematical model able to describe the time evolution of the different groups of phenolic compounds during maceration, could give some information about the evolution of the main processes involved in this fundamental phase of wine-making. Such information could be used to determine the best extraction time that would give the desired concentration of phenols in the must/wine (extraction yield) and the desired composition (extraction selectivity). In fact, the organoleptic characteristics of a wine are not only related to the total amount of phenols extracted but rather are mainly due to the different concentrations of the various phenolic groups.

Samples of must/wine, collected directly from fermentors used for the commercial production of wines, were analysed and the data were used to identify the mathematical algorithms that describe the evolution of phenols extracted from grape skin, as a function of maceration time, the varieties of grapes used, the fermentor employed (LEM and Ganimede) and the wine-making technology used (wine-pressing and pumping over).

MATERIAL AND METHODS

Experimental runs

The experimental runs were carried out using must from the pressing of two different red grape varieties (Merlot and Sangiovese) cultivated at "Poliziano", a farm located near Siena in central Italy (Tuscany). The Merlot and Sangiovese grapes were harvested on September 6 and October 5, 2001, respectively.

To fill the fermentors (80 and 100 hL) with approximately the same type of must, the grapes in each wagon coming from different parts of the vineyard were divided into equal portions based on the number of fermentors to be used. The Merlot and Sangiovese grapes were macerated for 13 and 20 days, respectively. The initial temperatures of the musts obtained after pressing were 27°C for Merlot and 21°C for Sangiovese. In both cases, $K_2S_2O_5$ (0.12 g/kg of grapes) was added, followed by selected yeast (0.25 g/kg of grapes; LSA Lallemand), and then, after 3 days, fermentation activators (0.30 g/kg of grapes; Lallemand nutrient) were added. A single stainless steel fermentor (LEM 80 hL, produced by Trecieffe S.n.c.) was used for the fermentation of the Merlot grapes, while a second fermentor was used (Ganimede 100 hL, produced by TEC-SIM S.r.l.) for the Sangiovese grapes. Both fermentors controlled the fermentation temperature by removing the heat produced during alcoholic fermentation by cold water flowing inside a heat exchanger. This was done to ensure a fermentation temperature in the 28°-30°C range. While the Ganimede fermentor is equipped with a specific apparatus which uses the CO_2 produced during fermentation to promote the dispersion of solid berry parts in the must/wine, the homogenisation of the mass and, in particular, the dispersion of the solid grape parts was carried out in the LEM fermentor by wine-pressing or pumping-over.

Each experimental run could be identified easily by looking at: the variety of vine (M = Merlot; S = Sangiovese), the fermentor (L = LEM; G = Ganimede) and the wine-making technology employed (P = wine-pressing, R= pumping over).

Chemical analysis

On each day of maceration (13 days for Merlot and 20 days for Sangiovese), a sample of must/wine was collected and analysed, according to the Official Methods of Analysis (A.O.A.C., 1984), to determine pH, total acidity, volatile acidity, reducing sugars, ethanol, malic acid, total and free sulphur dioxide and total dry matter. The analytical procedures

reported in the literature by DI STEFANO *et al.* (1989) and GLORIES (1999) were followed in order to evaluate the evolution of the concentrations of the different groups of phenolic compounds dissolved in the must/wine during the maceration of the solid berry parts.

The total content of phenolic compounds was determined by comparing the absorbance (λ = 280 nm) measured in a sample of must/wine (diluted 1/100 in a solution of 13% ethanol in water) with data from a calibration curve obtained using the absorbance values as a function of the trans-(+) catechin employed. The anthocyanins were evaluated by measuring the absorbance at 520 nm before and after treatment with sulphur dioxide (PARODI, 1999).

The vanillin assay was based on the ability of vanillin to react with catechin and small-sized oligomers (dimer, trimer, etc.) to give a coloured product. The differences in absorbance evaluated at 500 nm between two samples to which vanillin had been added or not were compared with the data from a calibration curve obtained using solutions of known concentrations of pure catechin. This comparison allowed the amount of flavanols, characterized by a low polymeric level, to be evaluated.

A specific statistical program (BUZZI FERRARIS and MANCA, 1996) was used to identify the best values to be assigned to the functional parameters of the mathematical equation used to relate the evolution of the data to extraction time. In this commercial program the minimum of function F, which is determined as the sum of the squares of the differences between calculated (R*calc,i) and experimental (R*calc,i) values, can be identified in a space of j-dimensions (where: j = number of equation parameters)

$$F = \sum_{i=1}^{N} (R^*_{calc.,i} - R^*_{exper.,i})^2$$

where: i = i-experimental value; N = total number of experimental values.

RESULTS AND DISCUSSION

Evolution of alcoholic fermentation during maceration of Sangiovese and Merlot grapes

The time evolution of ethanol accumulation during alcoholic fermentation is reported as a function of the fermentor used for Sangiovese and Merlot grapes (Fig. 1a,b), respectively. Data obtained

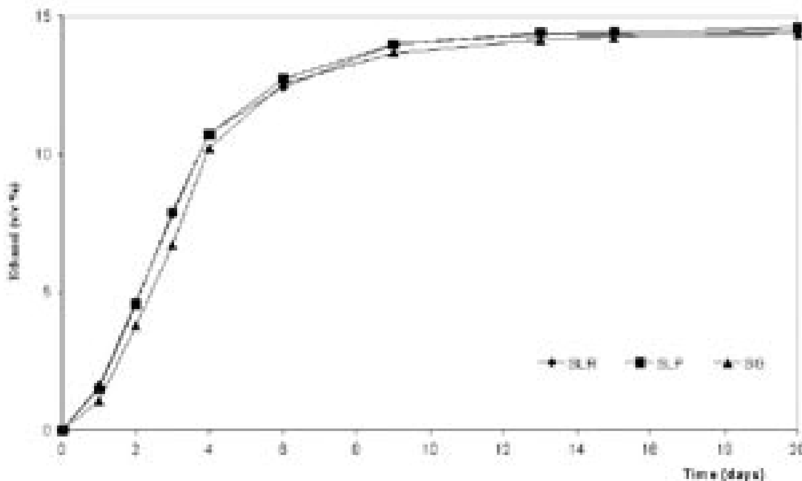

Fig. 1a - Trend of ethanol production during the fermentation of Sangiovese grapes (S) as a function of the fermentor (L = LEM; G = Ganimede) and the wine-making technology (P = wine-pressings, R = wine pumping-over).

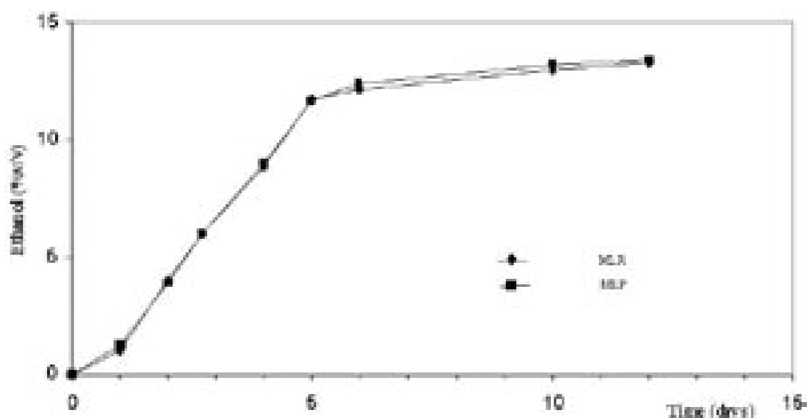

Fig. 1b - Time of ethanol production during the fermentation of Merlot grapes (M) using LEM (L) fermentor and as a function of the wine-making technology (P = wine-pressings, R = wine pumping-over).

using Sangiovese grapes, did not seem to be greatly affected by the wine-making technology (compare SLP with SLR in Fig. 1a) while an initial difference can be noted if the fermentor is changed (compare L = LEM; G = Ganimede). In any case, at the end of alcoholic fermentation, the three curves (SLP, SLR and SG) overlapped and similar asymptotic values were reached. Analogous conclusions can be deduced looking at the data obtained using Merlot grapes (Fig. 1b). In this case only one fermentor (L=LEM) was used and no significant differences can be noted between the two curves (MLP and MLR) obtained by changing the wine-making technology (P = wine-pressing, R = pumping over).

As reported in the literature (AMRANI and GLORIES, 1994; PARODI, 1999), phenol solubility is affected by the ethanol concentration accumulated inside the fermentor. In particular, when the alcohol concentration increases, the cuticle surrounding the grape seeds dissolves and the higher molecular weight tannins responsible for the sensation of astringency, are extracted. The composition of the solvent phase changed during the maceration of the grapes due to alcoholic fermentation; the data showed that the evolution over time was very similar, at least in the experimental runs carried out with grapes of the same variety.

Evolution of phenolic compounds during maceration of Sangiovese and Merlot grapes

The data (SLR) related to the concentrations of both total phenols and catechins (Fig. 2) appear to be closely associated with extraction time from the curve that passes through the origin of the axes and tends to an asymptotic value as extraction time increases (AMRANI and GLORIES, 1994; MORETTI, 1992). In contrast, the data related to anthocyanin extraction during the maceration of the solid grape parts, initially increased reaching a maximum and then decreased (Fig. 3). Two phenomena are therefore hypothesized to occur simultaneously during anthocyanin extraction: 1) the mass transfer of anthocyanins from the solid parts of the grapes into the liquid phase (must/wine) (KOVAK, 1978), which is the process responsible for anthocyanin accumulation in the liquid phase, and 2) the simultaneous and/or successive transformation of those anthocyanins into compounds capable of adsorbing in the visible region, particularly in the 520-540 nm range which is associated with their loss. These phenomena, responsible for the decrease in the anthocyanin concentration in the must/wine, may be more or less active depending on the working conditions adopted, as well as the wine-

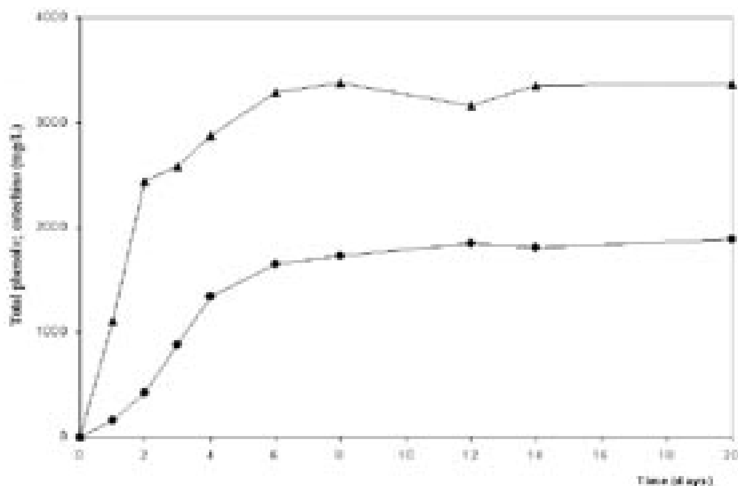

Fig. 2 - The typical trend of the total phenols (▲) and catechins (●) extracted from the liquid phase during the maceration of the solid grape parts (SLR).

making phase analysed (SINGLETON, 1987; SARNI *et al.*, 1995).

Among the many transformations which could potentially decrease the phenol concentration (oxidation; absorption inside inactivated yeasts; inclusion within the tartaric acid salt crystals; copolymerisation with other phenolic components; interaction with proteic substances, etc.) and because of the high reactivity shown by this group of phenols towards O_2 dissolved in the reaction medium, oxidation was assumed to be the main cause of the decrease of anthocyanins in the must/wines analysed during maceration (SINGLETON, 1987; GLORIES 1990). So, although a significant amount of the O_2 diffused into the must is consumed by the yeast for its metabolic activities, the remaining fraction would be sufficient to promote the oxidation of many compounds, including the anthocyanins dissolved in the must/wine.

Mathematical modelling of total phenol and catechin extraction

The mathematical equation, obtained by applying Fick's law to the diffusion that occurs between two heterogene-

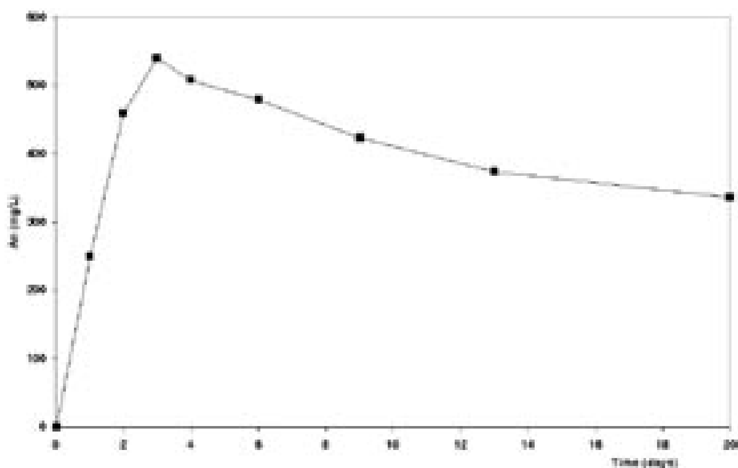

Fig. 3 - The typical trend of anthocyanin (An) accumulation in a must during the maceration of the solid grape parts (SLR).

ous phases (the solid grape part and the must/wine), was used to describe the evolution of total phenols and catechins with extraction time. The accumulation rate ($d[S]_{l,t=t}/dt$) of total phenols/catechins (S) in the liquid phase was supposed to be proportional to the difference between the concentration of extracted S ($[S^*]_{l,t=t}$), which would be in the must/wine if an equilibrium were reached between the amount of S still present inside the grape dregs ($[S]_{s,t=t}$), and that ($[S]_{l,t=t}$) of the S already extracted and that actually present in the liquid phase at the run time analysed:

$$d[S]_{l,t=t}/dt = k_s \cdot A \cdot ([S^*]_{l,t=t} - [S]_{l,t=t}) \quad \text{<eq. 1>}$$

where:

k_s = mass transfer constant ($s^{-1} \cdot m^{-2}$);

A = area of solid grape parts wet by must/wine (m^2).

If H is the equilibrium constant related to the diffusion of S between the two phases involved:

$$H = [S^*]_{l,t=t}/[S]_{s,t=t};\ [S^*]_{l,t=t} = H \cdot [S]_{s,t=t} = H \cdot ([S]_{s,t=0} - [S]_{l,t=t})$$

then equation <1> becomes:

$$d[S]_{l,t=t}/dt = k_s \cdot A \cdot ([S^*]_{l,t=t} - [S]_{l,t=t}) = k_s \cdot A \cdot (H \cdot ([S]_{s,t=0} - [S]_{l,t=t}) - [S]_{l,t=t}) = k_s \cdot A \cdot (H \cdot [S]_{s,t=0} - [S]_{l,t=t} \cdot (H + 1))$$

which, after mathematical integration, gives the following relation which describes the evolution of the accumulation of species S (total phenols/catechins) in the liquid phase:

$$[S]_{l,t=t} = H_s \cdot [S]_{s,t=0} / (H_s + 1) \cdot (1 - e^{-(H_s + 1) \cdot k_s \cdot A \cdot t}) \quad \text{<eq. 2>}$$

where H_s is the equilibrium constant (Henry) related to the diffusion of component S between the two heterogeneous phases analysed (solid phase = grape dregs and liquid phase = must/wine).

Moreover, according to the experimental evidence obtained, this function tends to an asymptotic maximum value as extraction time increases:

$$\lim_{t \to \infty} [S]_{l,t=t} = H_s \cdot [S]_{s,t=0} / (H_s + 1) = \text{constant} = [S]_{l,t=\infty}$$

and it becomes equal to zero at the beginning of maceration (t = 0), when no phenols have been extracted in the liquid phase:

$$\lim_{t \to 0} [S]_{l,t=t} = 0$$

Equation 2 was then transformed, to give:

$$[S]_{l,t=t} = [S]_{l,t=\infty} \cdot (1 - e^{-k_s^* \cdot t}) \quad \text{<eq. 3>}$$

where $k_s^* = (H_s + 1) \cdot k_s \cdot A$ = constant.

To identify the best numerical values to be assigned to the two functional parameters, $[S]_{l,t=\infty}$ and k_s^*, a specific statistical program was used (see Materials and Methods). The values found for $[S]_{l,t=\infty}$ and k_s^* and their related confidence intervals (c.i.; p=0.05) evaluated as a function of grape variety, fermentor and wine-making technology employed are reported in Tables 1 and 2 for total

Table 1 - Calculated values of the functional parameters (k_s^*, $[S]_{l,t=\infty}$) and confidence intervals (c. i.) in the equation used to relate the time evolution of the data for total phenols extracted in the liquid phase with the grape variety (S = Sangiovese; M = Merlot), fermentor (L = LEM; G = Ganimede) and wine-making technology (P = wine-pressing, R = wine pumping-over).

Run	k_s^* ± c.i.	$[S]_{l,t=\infty}$ ± c.i.	r
SLP	0.5269 ± 0.0002	3,335.9 ± 0.4	0.98
SLR	0.4901 ± 0.0002	3,193.4 ± 0.4	0.94
SG	0.3065 ± 0.0001	3,518.6 ± 0.5	0.99
MLP	0.7487 ± 0.0037	2,551.1 ± 3.1	0.92
MLR	0.5660 ± 0.0030	2,616.5 ± 3.4	0.93

Table 2 - Calculated values of the functional parameters involved in the equation used to relate the time evolution of the data for catechins extracted in the liquid phase with the grape variety (S = Sangiovese; M = Merlot), fermentor (L = LEM; G = Ganimede) and wine-making technology (P = wine-pressing, R= wine pumping-over).

Run	k_S^* ± c.i.	$[S]_{l,t=\infty}$ ± c.i.	r
SLP	0.2143 ± 0.0002	2,041.7 ± 0.7	0.93
SLR	0.1883 ± 0.0002	1,970.6 ± 0.7	0.95
SG	0.1920 ± 0.0002	2,107.2 ± 0.7	0.93
MLP	0.2439 ± 0.0028	1,453.9 ± 6.3	0.93
MLR	0.2644 ± 0.0029	1,524.4 ± 5.7	0.94

phenols and catechins, respectively. The high correlation coefficient values, calculated for the linear form of the mathematical equation employed (eq. 3), give a measure of the suitability of the mathematical equation used to describe the evolution of total phenols and catechins with maceration time. Since $1/k_s^*$ can be assumed to be a valid measure of the resistance caused by dregs to the diffusion of these compounds into the liquid phase, their concentration in the grapes can be evaluated by $[S]_{l,t=\infty}$.

According to data reported in Table 2, the catechin concentration in Sangiovese grapes appears to be greater than that in Merlot ($[S]_{l,t=\infty}$ of Sangiovese ≈ 2,000, while that of Merlot ≈ 1,500 mg/L); the resistance of the Sangiovese dregs (≈ 5 days) is also greater than that of Merlot (≈ 4 days).

The same considerations apply to the results obtained for total phenols (Table 1) but, in this case, the data show a greater variability, particularly with respect to Merlot grapes. In any case, Sangiovese grapes appear to be richer in phenolic compounds than Merlot grapes but it is much easier to extract these compounds from Merlot grapes than from Sangiovese grapes.

Moreover, neither fermentor (LEM and Ganimede) nor wine-making technology (wine-pressing and pumping over) seem to markedly affect the amount and rate of catechin and total phenol extraction. In fact, the differences among the data obtained (Tables 1 and 2) could not be correlated to the different apparatus or methodology used.

Mathematical modelling of anthocyanin extraction and oxidation

Assuming that oxidation is the main cause for the decrease of anthocyanins in the must/wines during maceration, O_2 diffusion between the gaseous (air) and liquid phases (must) was also taken into account, to describe the main phenomena concerning phenol evolution during grape maceration.

Anthocyanin extraction

To describe the time evolution of anthocyanin diffusion rates ($R_{mt,An}$) and of O_2 ($R_{mt,O2}$) in the must/wine, the same kinetic equation (eq. 1) adopted for catechins and total phenols was used:

$$R_{mt,An} = k_{An} \cdot A \cdot ([An^*]_{l,t=t} - [An]_{l,t=t}) \quad \text{<eq. 4>}$$

where:

$[An^*]_{l,t=t}$ = concentration of anthocyanins dissolved in the liquid phase which would be in equilibrium (H = equilibrium constant = $[An^*]_{l,t=t}$ / $[An]_{s,t=t}$) with the amount of anthocyanins still present inside the grapes ($[An]_{s,t=t}$); $[An^*]_{l,t=t} = H \cdot [An]_{s,t=t}$ ($g \cdot L^{-1}$);

$[An]_{l,t=t}$ = concentration of anthocyanins in the liquid phase at a random time t=t ($g \cdot L^{-1}$);

k_{An} = mass transfer constant ($s^{-1} \cdot m^{-2}$);

A = area of contact occurring between the two phases involved: solid grape parts and must/wine (m^2).

The amount of anthocyanins diffused into the liquid phase, which is given by the difference between the concentration initially present in the solid part of

the grapes ($[An]_{s,t=0}$), when no anthocyanins have been extracted into the liquid phase, and that present at a random time t=t ($[An]_{s,t=t}$), should be equal to the sum of the concentrations of anthocyanins still present in the liquid phase ($[An]_{l,t=t}$) and that of the product already oxidised ($[An]_{ox,t=t}$):

$$[An]_{s,t=0} - [An]_{s,t=t} = [An]_{l,t=t} + [An]_{ox,t=t}$$

Assuming $[An]_{ox,t=t}$ to be negligible, if compared to the amount of anthocyanins dissolved in the must $[An]_{l,t=t}$ at a random time t=t, the following relationship can be obtained:

$$[An]_{s,\ t=t} \approx [An]_{s,t=0} - [An]_{l,t=t}$$

so that:

$$[An^*]_{l,t=t} = H \cdot [An]_{s,t=t} = = H \cdot ([An]_{s,t=0} - [An]_{l,t=t}) \quad \text{<eq. 5>}$$

Combining eq. 4 and 5 it is possible to obtain:

$$R_{mt,An} = k_{An} \cdot A \cdot ([An^*]_{l,t=t} - [An]_{l,t=t}) = = k_{An} \cdot A \cdot (H \cdot ([An]_{s,t=0} - [An]_{l,t=t}) - [An]_{l,t=t}) = = k_{An} \cdot A \cdot (H \cdot [An]_{s,t=0} - [An]_{l,t=t} \cdot (H+1)) = = k_{An} \cdot A \cdot H \cdot [An]_{s,t=0} + - k_{An} \cdot A \cdot (H+1) \cdot [An]_{l,t=t} \quad \text{<eq. 6>}$$

as:

$$k_{An} \cdot A \cdot H \cdot [An]_{s,t=0} = \text{constant} = = k_{1,An} \text{ and } k_{An} \cdot A \cdot (H+1) = = \text{constant} = k_{2,An},$$

eq. 6 becomes:

$$R_{mt,An} = k_{1,An} - k_{2,An} \cdot [An]_{l,t=t} \quad \text{<eq. 7>}$$

O_2 diffusion

The same approach adopted for the anthocyanins was followed to describe O_2 diffusion between the surrounding atmosphere and the liquid phase (must/wine):

$$R_{mt,O2} = = k_{O_2} \cdot A_{O_2} \cdot ([O_2^*]_{l,t=t} - [O_2]_{l,t=t}) \quad \text{<eq. 8>}$$

where:

$[O_2^*]_{l,t=t}$ = concentration of O_2 dissolved in the liquid phase, at the generic time t=t, if the saturation equilibrium (H_{O2} = saturation constant = $[O_2^*]_{l,t=t} / PO_2$) with the external atmosphere (PO_2 = constant = 20.8 kPa) were reached ($g \cdot L^{-1}$);

$[O_2]_{l,t=t}$ = concentration of O_2 dissolved and really present in the liquid phase, at a random time t=t ($g \cdot L^{-1}$);

k_{O_2} = mass transfer constant ($s^{-1} \cdot m^{-2}$);

A_{O_2} = area of contact occurring between the two involved phases: surrounding atmosphere and must/wine (m^2).

So eq. 8 could be transformed:

$$R_{mt,O2} = k_{O_2} \cdot A_{O_2} \cdot ([O_2^*]_{l,t=t} - [O_2]_{l,t=t}) = = k_{O_2} \cdot A_{O_2} \cdot (PO_2 \cdot H_{O_2} - [O_2]_{l,t=t}) = = k_{O_2} \cdot A_{O_2} \cdot PO_2 \cdot H_{O_2} - k_{O_2} \cdot A_{O_2} \cdot [O_2]_{l,t=t} = = k_{4,O_2} - k_{5,O_2} \cdot [O_2]_{l,t=t} \quad \text{<eq. 9>}$$

where $k_{O_2} \cdot A_{O_2} \cdot PO_2 \cdot H_{O2}$ = constant = $k_{4,O2}$ and $k_{O2} \cdot A_{O2}$ = constant = $k_{5,O2}$

Anthocyanin oxidation

The rate of anthocyanin oxidation ($R_{ox,An}$) at random time t=t; was thought to be directly proportional (k_3) to the concentrations of anthocyanins ($[An]_{l,t=t}$) and oxygen ($[O_2]_{l,t=t}$) dissolved in the liquid phase:

$$R_{ox,An} = k_3 \cdot [An]_{l,t=t} \cdot [O_2]_{l,t=t} \quad \text{<eq. 10>}$$

Evolution of anthocyanin concentration with maceration time

The time evolution of anthocyanin and O_2 dissolved in the liquid phase during maceration could therefore be evaluated as the difference between the rates related to their accumulation ($R_{mt,An}$ and R_{mt,O_2}) and those connected with their consumption ($R_{ox,An}$). The following system of five differential equations was obtained:

1) $R_{mt,An} = k_{1,An} - k_{2,An} \cdot [An]_{l,t=t}$ <eq. 7>
2) $R_{mt,O_2} = k_{4,O_2} - k_{5,O_2} \cdot [O_2]_{l,t=t}$ <eq. 9>
3) $R_{ox,An} = k_3 \cdot [An]_{l,t=t} \cdot [O_2]_{l,t=t}$ <eq. 10>

4) $d[An]_{t=t}/dt = R_{mt,An} - R_{ox,An}$ <eq. 11>

5) $d[O_2]_{t=t}/dt = R_{mt,O_2} - R_{ox,An}$ <eq. 12>

Although many other processes could promote O_2 consumption in the must/wine, such as the energy production necessary for yeasts and the oxidation of many other different compounds dissolved in the must/wine, this preliminary, simplified model was initially tested.

To identify the best values (Table 3) to be assigned to the mathematical parameters ($k_{1,An}$; $k_{2,An}$; k_{4,O_2}; k_{5,O_2}; k_3) involved in these equations, a specific statistical program (Burenl) was used (BUZZI FERRARIS and MANCA, 1996). Moreover, the numerical integration (WORTING and GEFFNER, 1965) of the differential equations obtained, was used to describe the time evolution of $[An]_{1,t=t}$ and $[O_2]_{1,t=t}$.

Despite the marked, rough simplifications adopted to formulate this model, the high correlation coefficient values and the good degree of overlapping between experimental and calculated values appear to test the suitability and reliability of the equations introduced. As previously reported, the mathematical parameters involved in the model were obtained as the product of many constants and cannot be assumed to represent a specific physical characteristic. Nevertheless, in some cases, it was possible to identify the process or the physical parameter to which they appear to be more strongly related. So $k_{1,An}$ can be assumed to be a valid measure of the "maximum amount of anthocyanins that could be potentially extracted" from those particular grapes and a vine having a high $k_{1,An}$ value would have a high fraction of extractable anthocyanins. The Merlot grape is characterised by a higher anthocyanin content than Sangiovese ($k_{1,An}$ values) and these phenolic compounds can also be extracted more easily from Merlot grapes than from Sangiovese grapes (compare the $k_{2,An}$ values). On the contrary, the reactivity of the anthocyanins with dissolved O_2, does not seem to be affected by the type of grapes used during maceration, as shown by the k_3 constant values which do not change significantly as a function of the grape. Moreover, the parameters related to O_2 diffusion, the k_{4,O_2} (a measure of O_2 diffused into the liquid phase when the saturation equilibrium is reached) and k_{5,O_2} constants (a parameter related to O_2 mass transfer rate) do not vary significantly with the variety of

Table 3 - Calculated values of the functional parameters involved in the system of differential equations used to relate the time evolution of the data of anthocyanins in the liquid phase with the grape variety (S = Sangiovese; M = Merlot), fermentor (L = LEM; G = Ganimede) and wine-making technology (P = wine-pressings, R= wine pumping-over).

Functional parameter related to:	SLP	SLR	SG	MLP	MLR
Maximum amount of anthocyanins that could potentially be extracted ($k_{1,An}$)	$5.00 \cdot 10^{-1}$	$4.50 \cdot 10^{-1}$	$4.40 \cdot 10^{-1}$	2.15	2.15
Tendency of anthocyanins to be extracted in the liquid phase ($k_{2,An}$)	$1.1 \cdot 10^{-7}$	$2.3 \cdot 10^{-7}$	$6.1 \cdot 10^{-7}$	$7.2 \cdot 10^{-1}$	$9.9 \cdot 10^{-1}$
Reactivity of anthocyanins with dissolved O_2 (k_3)	$12.8 \cdot 10^{-3}$	$8.1 \cdot 10^{-3}$	$10.5 \cdot 10^{-3}$	$10.6 \cdot 10^{-3}$	$2.4 \cdot 10^{-3}$
Amount of O_2 dissolved in the liquid phase at saturation ($k_{4,O2}$)	7.10	7.01	7.09	7.13	7.20
Tendency of O_2 to dissolve in the liquid phase ($k_{5,O2}$)	$14.1 \cdot 10^{-2}$	$7.8 \cdot 10^{-2}$	$1.6 \cdot 10^{-2}$	$6.9 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$
Correlation coefficient evaluated for the linearized form	0.93	0.97	0.96	0.99	0.91

grapes used. As already reported for total phenols and catechins, the anthocyanin diffusion data, although affected by a more consistent variability, do not seem to change significantly with the fermentor and/or the wine-making technology adopted.

Mathematical modelling of the extraction and oxidation of other phenolic compounds

Due to the low number of samples collected and the few experimental points available for Merlot grapes, the following mathematical elaboration was carried out using Sangiovese data. Fig. 4 reports the trend with maceration time of the differences between the experimental data related to total phenols ($P_{tot,\ t=t}$) and the sum of those found for catechins ($Cat_{t=t}$) and anthocyanins ($An_{t=t}$):

$$[P_{tot}]_{t=t} - ([Cat]_{t=t} + [An]_{t=t}) = = [P_A]_{t=t} + [P_B]_{t=t}$$

This particular trend, observed in all three experimental runs involving Sangiovese grapes (SLP, SLR and SG), was hypothesized to be due to the sum of two different groups of phenols extracted in the liquid phase: P_A (group A) which appears to be easily extracted from the grape but is also rapidly converted and lost (probably oxidised) and P_B (phenols of group B) which took a long time to diffuse into the liquid phase but appear to be particularly stable. So, an empirical equation was used to describe the evolution of the P_A group with maceration time:

$$[P_A]_{t=t} = C_3 \cdot e^{\,t \cdot (-C1 \cdot t + C2)}$$

the same mathematical form used to relate the time evolution of total phenols and catechins was also used to describe that of the P_B group (eq. 3):

$$[P_{B,}]_{1,t=t} = [P_B]_{1,t=\infty} \cdot (1 - e^{-k^*_B \cdot t})$$

Table 4 reports the constants introduced into the equations used and calculated by the statistical program (BURENL). The good degree of overlapping that occurred between the experimen-

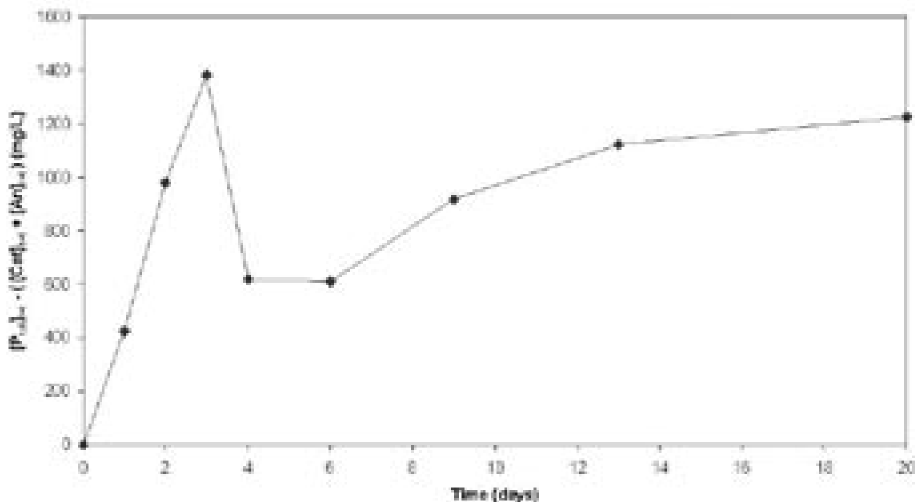

Fig. 4 - The trend of the differences between the experimental data related to the total phenols and the sum of catechins and anthocyanins, with maceration time (SLR).

Table 4 - Calculated values of the functional parameters involved in the equations used to relate the evolution of the differences between the data related to total phenols and the sum of catechins and anthocyanins extracted from Sangiovese grapes as a function of fermentor (L = LEM; G = Ganimede) and wine-making technology (P = wine-pressings, R= wine pumping-over).

Functional parameter related to:	SLP	SLR	SG
Reactivity of P_A with dissolved O_2 (C_1)	$4.79 \cdot 10^{-1}$	$5.23 \cdot 10^{-1}$	$6.94 \cdot 10^{-1}$
Tendency of P_A to be extracted in the liquid phase (C_2)	2.26	2.76	3.59
Concentration of P_A initially present in the liquid phase (C_3)	$9.39 \cdot 10^{1}$	$3.78 \cdot 10^{1}$	$1.00 \cdot 10^{1}$
Asymptotic maximum value related to P_B mass transfer (C_4)	$1.46 \cdot 10^{3}$	$1.43 \cdot 10^{3}$	$1.48 \cdot 10^{3}$
Tendency of P_B to dissolve in the liquid phase (C_5)	$9.99 \cdot 10^{-2}$	$9.99 \cdot 10^{-2}$	$9.73 \cdot 10^{-2}$
Correlation coefficient evaluated for the linearized form	0.92	0.79	0.97

tal and calculated values and the high correlation coefficients seem to confirm the hypothesis.

As shown by the C_3 values, the initial concentration assumed by the P_A group seems to vary greatly depending on the experimental conditions. Moreover, the P_A fraction, which is characterised by a high solubility in the reaction medium and a strong tendency to be oxidised, is very sensitive to the working conditions. The ability of the P_A fraction to diffuse quickly and react, does not seem to be significantly affected by the apparatus or the wine-making technology (compare C_1 and C_2 data).

In contrast, a very low variability appears to affect the values connected with the asymptotic maximum (C_4) of the P_B extraction curve, as well as that of C_5 which describes the tendency of this phenolic group to diffuse into the liquid phase. So, on the basis of these results, the P_A group was hypothesized to be made up of low molecular weight phenolic compounds, characterised by a high solubility and reactivity with the O_2 dissolved in the extraction medium, to produce more oxidised compounds (quinones). This group could be made up of derivatives of benzoic and/or cinnamic acids which may be significantly present in the harvested grapes and then decrease quickly during maceration (MORETTI, 1992).

On the contrary, the P_B group could be made up of colourless (no absorption in the 520-530 nm region) high molecular weight phenols. These compounds could be tannins, that are more complex than catechins, characterised by a high degree of polymeration or located in some parts of the grape (seeds) that are not easily reached by the extraction solvent (must/wine).

To measure the suitability of the mathematical model introduced, the theoretical evolution of the total phenols (P_{tot}) and of the other phenolic groups (P_A= derivatives of benzoic and/or cinnamic acids; An = anthocyanins; P_B = high-molecular weight tannins; Cat = catechins) was compared with the experimental data collected (Fig. 5). Moreover this model was used to describe the evolution of phenolic compounds during the cold maceration of Sangiovese grapes (~7°C) and to compare these experimental data with those obtained working at 21°C (ANDRICH *et al.*, 2004). When the temperature decreased, the rates of anthocyanin diffusion and their successive oxidation in the liquid phase also decreased. The anthocyanin oxidation rate in the liquid phase appears to be more affected by temperature than extraction rate and the wine macerated at 7°C had more colour and a more pleasant taste than that produced at 21°C.

Using the mathematical equations that can describe the evolution of the total phenols as well as that of the different phenolic groups, it is possible to evalu-

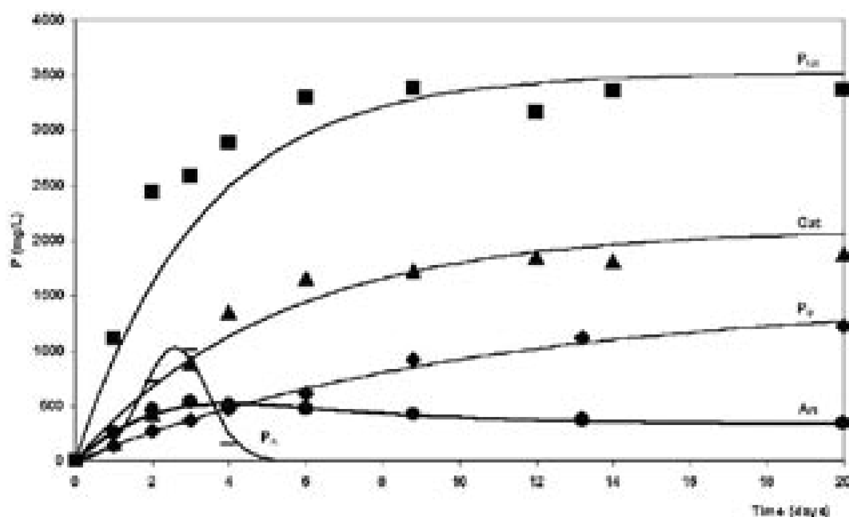

Fig. 5 - The theoretical trend of total phenols (■ P_{tot}) and of the different phenolic groups (▬ P_A, ● An, ◆ P_B, ▲ Cat) with maceration time of Sangiovese grapes (SLR).

ate the extraction yield and the composition of this extract (extraction selectivity) as a function of the maceration time. Table 5 reports the time evolution of the ratio ($P_{tot,\ t=t}/P_{tot,\ t=\infty} \cdot 100$) between the total phenols extracted at a random time t=t, and the total amount of these compounds which could potentially be extracted (extraction yield, $P_{tot,\ t=\infty}$) using very long maceration times ($t = \infty$). After one day of maceration, only 26% of the extractable phenols were diffused into the liquid phase; this percentage increased rapidly to 80% after about 5 days of maceration and then did not change significantly from 14 to 20 days.

During the initial phase of maceration the extract was particularly rich in phenols belonging to the P_A group (benzoic and cinnamic acids derivatives) and anthocyanin group. As the extraction time increased, their concentrations in the liquid phase (must/wine) rapidly decreased particularly the concentration of the P_A compounds, while the percentages of catechins and P_B com-

Table 5 - Extraction yield and extract composition as a function of maceration time (calculated using parameters from SG run).

Maceration time (days)	1	5	12	14	20
Extraction yield					
$P_{tot,\ t=t} / P_{tot,\ t=\infty} \cdot 100$	26.4	78.4	97.5	99.7	99.8
Extract composition					
P_A (%)	19.2	0.7	~0	~0	~0
Catechins (%)	38.6	54.1	56.4	55.9	56.2
Anthocyanins (%)	28.0	21.2	13.3	10.2	9.2
P_B (%)	14.2	24.0	30.3	31.3	34.6

pounds (higher-molecular weight tannins or phenols located in areas not easily reached by the must/wine) increased steadily. Moreover, after 14 days of maceration the chemical composition of extracts did not seem to vary significantly with extraction time.

CONCLUSIONS

This mathematical model can be used to determine the maceration time that should be adopted to produce a wine having a desired phenolic composition. To produce a wine that is rich in colour, low in tannins, with a high degree of polymerisation and is ready to be consumed in a short time, it is better to reduce the extraction time. In contrast, a longer maceration time is required to obtain a more structured, complex wine that can be kept in oak barrels and barriques for a long time. According to the mathematical model introduced, increasing the maceration time by more than two weeks does not result in any further accumulation of total phenols in the liquid phase and the composition of the extract does not vary significantly. As the contact time between the liquid and solid parts of the grapes increases, so does the possibility that the wine can take on undesirable flavours and tastes (less flavour and taste). In order to extract other compounds like mannoproteins from inactivated yeasts, the wine has to be in contact with the fermentation dregs for a long time.

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PAPER

STUDY OF AMARONE VALPOLICELLA WINE AGEING USING CHEMICAL PARAMETERS

STUDIO DELL'INVECCHIAMENTO DEL VINO AMARONE DELLA VALPOLICELLA ATTRAVERSO PARAMETRI CHIMICI

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ABSTRACT

The evolution of the chemical and physical parameters of Amarone Valpolicella wine samples was followed during various stages of production. In addition to many basic parameters, the polyphenolic constituents were evaluated by HPLC. The values showed a wide range until the end of alcoholic fermentation, and then tended to level off once the product was casked for ageing. The data were analysed using Partial Least Squares Regression (PLS). It was found that pH, total polyphenols and total an-

RIASSUNTO

In questo lavoro è stata seguita l'evoluzione di parametri chimico-fisici del vino Amarone della Valpolicella in diverse fasi produttive. Oltre a molti parametri di base, sono stati valutati i costituenti polifenolici con analisi HPLC. Fino alla conclusione della fermentazione alcolica, i valori sono compresi in un ampio intervallo; successivamente tendono a livellarsi una volta che il prodotto è posto ad affinare in botte. Tutti i dati ottenuti sono stati trattati statisticamente applicando il Partial Least

- Key words: Amarone, chemical parameters, PLS, polyphenols, wine ageing -

thocyanins, gallic acid, catechin, p-coumaric acid, *t*-resveratrol and cyanidin-3-O-glucoside content were significantly related to wine ageing.

Squares Regression (PLS). È risultato che i parametri più correlati all'invecchiamento del vino sono pH, antociani e polifenoli totali, acido gallico, catechina, acido *p*-cumarico, *t*-resveratrolo e cianidina 3-glucoside.

INTRODUCTION

The wine market throughout the world is developing to satisfy consumers, who are increasingly looking for high quality products with the tastes and fragrances of lost traditions. Many wine-producing zones in the world are famous for offering some kind of "passito", like Tokaj from Hungary, Sauternes from France, Xeres from Spain, and more recently, Sherry from California; in these cases, the grapes are allowed to dry either naturally or under forced conditions.

Amarone della Valpolicella is a very old wine that was already known to the soldiers of the Roman emperor Caesar Augustus, who began using a drying technique in the area near Verona to produce wines with a high alcohol content (GUY, 2000). Amarone Valpolicella wine thus has a two-thousand year old tradition, according to which grape bunches are spread over trellis racks in barns and left to dry for months. The wine is subsequently left in cellars for four years (3 years in casks, followed by 1 year in bottles). All of these steps are undoubtedly important and contribute to making this wine with its particular taste well known and appreciated throughout the world.

The development of this wine during the ageing phase has recently become a subject of interest for researchers, who hope to improve production techniques and thus ensure a high quality product that keeps well. Many factors such as temperature, sun exposure and soil composition, greatly affect the features of a wine at the end of ageing (KLIEWER, 1977; ANDREAS-LACUEVA *et al.*, 1997; BARBEAU *et al.*, 2001). In particular, the ageing of a wine depends on a number of chemical and physical parameters, of which color (BAKKER and TIMBERLAKE, 1986; HEREDIA and TRONCOSO, 1997) and anthocyanin content (BAKKER, 1986; MATEUS *et al.*, 2003) are of primary importance.

In this paper, we report the evolution of several chemical and physical parameters of samples taken at the various steps of production of Amarone wine obtained from the 1998-2001 vintages. Since the law specifies that the wine can be marketed four years after vintage, the evolution of the physical and chemical characteristics were determined from the time of grape harvesting to the end of the cellar life. The great homogeneity of the starting materials, obtained by carefully controlling the growing conditions, the period of harvesting, withering (computer controlled, Technical Group Masi, personal communication) grape mixing before pressing, and finally, the standardization of the production cycle, achieved after many years of practice, allowed us to assume that the wines were similar. Consequently, it was hypothesized that if the evolution of the single parameters in the various ageing phases were followed, it should be possible to carry out easy analyses in a timely manner that would subsequently help producers predict the possibility of obtaining quality wines. Moreover, some samples from earlier years, chosen from

those that experts have defined as the best (BURTON, 2000), were evaluated in order to confirm the hypothesis.

MATERIALS AND METHODS

Samples

Samplings were carried out on Amarone must and wine from four different years, in different production phases. The same mixture of grapes, Corvina (70%), Molinara (5%) and Rondinella (25%), in accordance with legal requirements, was used in all of the years (G.U., 1990). As far as must (obtained from grapes of the year 2001) was concerned, samples were tested at five subsequent production steps: at the end of pressing (sample A), at the beginning of alcoholic fermentation (sample B), after 20 days of fermentation (samples C), after 40 days of fermentation, (samples D) and at the end of fermentation, (sample E).

Regarding the wine, Amarone samples from the years 1998, 1999, 2000 and 2001 were taken during different phases of the ageing process: in cask (225 L barriques made of Slavonia oak) for wines of the years 2001, 2000 and 1999 (samples F, G and H) and in bottle, 1998 (sample I). All samples were in a bulk obtained from about 40 single samples (Table 1).

In addition, four wines from 1997, 1990, 1988 and 1985, considered to be among the best Amarone wines on the market, were also analyzed.

Chemical standards

HPLC standards were gallic, caffeic and *p*-coumaric, (+)-catechin and rutin; quercetin and *trans*-resveratrol (Sigma-Aldrich srl, Milan, Italy); quercetin 3-*O*-glucoside, (-)-epicatechin and malvidin 3-*O*-glucoside were obtained from Extrasynthèse (Genay Cedex, France).

Analytical methods

For all the samples pH, volatile and titratable acidity, alcohol and residual sugar were determined using the FT-NIR (Fourier Transform Infrared Spectroscopy, WineScan FT 120, FOSS A/S, Denmark) technique, which allows rapid analysis of the wine (GISHEN and DEMBERGS 1998; MANLEY *et al.*, 2001).

Table 1 - Description of the samples analysed. Each sample was a bulk derived from the whole production.

Sample	Step of production	Features	Vintage year
A	Grape juice	Samples collected from just pressed grapes	2001
B	$Must_0$	Sample collected at the beginning of alcoholic fermentation, following the addition of yeast and activators	2001
C	$Must_{20}$	Must collected after 20 days of fermentation	2001
D	$Must_{40}$	Must collected after 40 days of fermentation, following the removal of skins	2001
E	$Must_F$	Must collected at the end of fermentation, ready to be casked	2001
F	Amarone wine 2001	One-month aged wine (casked)	2001
G	Amarone wine 2000	Thirteen-month aged wine (casked)	2000
H	Amarone wine1999	Twenty-six-month aged wine (casked)	1999
I	Amarone wine1998	Bottled wine ready to be marketed after twelve months in cellar	1998

Spectrophotometric analyses

A Hewlett-Packard 8453 spectrophotometer (Agilent Technologies Italia S.p.A., Milan, Italy) was used to measure total polyphenolic and total anthocyanin content, colour intensity and colour tonality. Total polyphenol content was assayed using the Folin reagent (DI STEFANO *et al.,* 1989). Total anthocyanins, expressed as mg/L of malvidin monoglucoside, were measured as described by DI STEFANO *et al.* (1989). The values for colour intensity and tonality were obtained following the official Italian method of wine analysis (G.U.,1986). All analytical parameters were in triplicate.

HPLC analyses

A Hewlett-Packard 1090 DAD HPLC apparatus equipped with an HP autoinjector, with ChemStation software was used, with a Supelco LC 18 reverse phase column (250x4.6 mm) (Sigma-Aldrich srl, Milan, Italy) run at 0.8 mL min^{-1} at room temperature. Elution was carried out using a mixture of eluent A (8.9% formic acid in water) and B (8.9% formic acid in 50% aqueous methanol). The elution profile was as follows: 0 min 75% A, 15 min 55% A, 50 min 1% A, 55 min 1% A, 58 min 75% A, 68 min 75% A. Samples were filtered on HA 0.45 µm Millipore filter (Millipore Italia SpA, Milan, Italy) and directly injected. Gallic acid, catechin and epicatechin were read at 280 nm; caffeic and *p*-coumaric acids and *t*-resveratrol at 320 nm; quercetin-3-O-glucoside, quercetin and rutin (expressed as quercetin) were read at 365 nm; anthocyanins were read at 520 nm and expressed as malvidin-3-O-glucoside. The phenol concentration in each sample was the mean of three replicates.

Statistical methods

All data obtained by chemical analysis were treated with a multivariate linear regression using the Partial Least Square (PLS) method, applying the statistical program Unscrambler® 8.0 (Camo As, Trodheim, Norway). The PLS method allows identification of the independent variables considered (all the analyzed chemical parameters) that most affect the dependent variable. The ageing time of the wine was considered as the dependent variable.

RESULTS AND DISCUSSION

To estimate the evolution of wine in the different production and ageing phases, an analytical characterization of the samples was carried out on the must and wine. The values obtained for the basic parameters measured in the samples using the Winscan FT 120 technique (Table 2) show that the greatest variation in the chemical parameters occurred during the first part of fermentation, with a sharp decrease in sugars and a subsequent increase in both the alcohol level, as well as the level of volatile acidity, which however remained well below legally established limits (G.U., 1986).

Moreover, the data obtained for the wines already casked (samples F, G and H) and bottled (sample I) show an excellent stability clearly meeting the quality standards required by the Amarone Valpolicella DOC Production Board (G.U., 1990). More information on the evolution of the product during the ageing phase can be obtained from an estimation of the spectrophotometric variables of the Amarone wine reported in Table 3, such as colour intensity and tonality, total anthocyanins and total polyphenols. The total anthocyanins increased during fermentation (samples A-D), because the must remained in contact with the skins during this forty-day phase. Subsequently, this value decreased sharply, probably due to polymerization (samples F-I) (BAKKER, 1986; BROUILLARD and DANGLES, 1994; MAZZA, 1995). To-

Table 2 - Results of the FTNIR analysis carried out on samples of Amarone wine. Must t_0, t_{20}, t_{40} and t_f = must at the beginning of fermentation, at the 20th day, 40th day and at the end of fermentation, respectively; n.d. = not determined.

	Sample	pH	Volatile acidity g/L	Titratable acidity g/L	Reducing sugars g/L	Alcohol % v/v
A	Grape juice	3.94±0.01	n.d.	5.18±0.01	25.7±0.2	n.d.
B	Must (t_0)	3.93±0.01	n.d.	4.45±0.02	25.7±0.1	n.d.
C	Must (t_{20})	3.82±0.03	0.33±0.04	5.71±0.03	1.78±0.01	14.62±0.19
D	Must (t_{40})	3.81±0.02	0.35±0.02	6.18±0.07	1.35±0.04	15.99±0.04
E	Must (t_f).	3.62±0.04	0.39±0.02	6.74±0.09	1.29±0.03	16.03±0.03
F	Wine, 2001 vintage	3.40±0.01	0.52±0.01	6.42±0.02	0.63±0.01	15.89±0.01
G	Wine, 2000 vintage	3.39±0.02	0.64±0.02	5.75±0.03	0.51±0.04	15.86±0.03
H	Wine, 1999 vintage	3.47±0.02	0.64±0.02	6.04±0.02	0.48±0.03	15.12±0.04
I	Wine, 1998 vintage	3.35±0.02	0.62±0.02	5.60±0.03	0.30±0.03	15.14±0.04

tal polyphenols had a linear tendency, increasing until the end of fermentation (sample F).

Once casked and bottled, the wine undergoes an ageing phase during which time different reactions take place. Since Amarone wine is aged four years before marketing, it is of primary importance to know whether the fresh wine (or at least the wine obtained during the first months of ageing in casks) has the characteristics required to go through the remaining ageing period so that, at the end of that period, the wine will have fully developed the required properties.

Since the total polyphenol content is the main and simplest parameter, it was studied in the different samples. The values reported in Table 3 as well as those measured in the samples of the best bottled wine from the last fifteen years are plotted in Fig. 1. It can be clearly seen that the phenolic content continuously decreases with ageing and, the data show a linear correlation between the concentration of total polyphenols and their decrease over time.

Therefore, after a short time, by the end of fermentation, for example, it should be possible to estimate wheth-

Table 3 - Results of the spectrophotometric analysis carried out on samples of Amarone wine. Must t_0, t_{20}, t_{40} and t_f = must at the beginning of fermentation, at the 20th day, 40th day and at the end of the fermentation, respectively; total polyphenols and anthocyanins are expressed as mg L^{-1}, of gallic acid and malvidin, respectively; n.d. = not determined.

	Sample	Colour intensity	Colour tonality	Total anthocyanins	Total polyphenols
A	Grape juice	0.84±0.03	n.d.	86.92±2.61	356.5± 6.4
B	Must (t_0)	2.03±0.06	n.d.	266.05±6.01	652.2±1.3
C	Must (t_{20})	8.35±0.21	0.67±0.03	331.87±5.79	1170.9±23.9
D	Must (t_{40})	7.95±0.11	0.64±0.02	348.88±2.45	1485.2±3.9
E	Must (t_f).	8.11±0.10	0.63±0.02	343.23±1.39	1621.9± 9.4
F	Wine, 2001 vintage	13.78±0.10	0.83±0.04	98.64±8.43	2801.8±14.9
G	Wine, 2000 vintage	9.75±0.08	0.85±0.04	78.88±1.57	2673.3±12.3
H	Wine, 1999 vintage	12.21±0.11	0.76±0.04	65.73±3.44	2602.2±9.9
I	Wine, 1998 vintage	10.77±0.16	0.71±0.04	55.32±2.77	2567.0±8.7

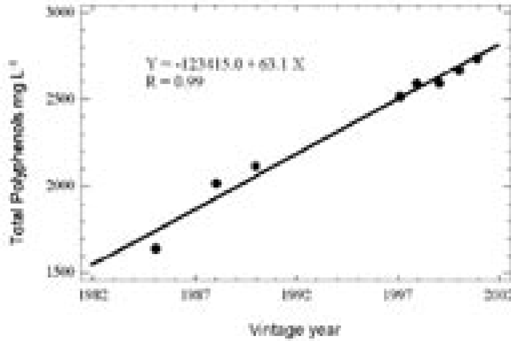

Fig. 1 - Regression curve for the values of total polyphenol versus vintage years of Amarone Valpolicella wine.

er or not the quality characteristics of the wine are likely to be preserved over time.

Some of the phenolic compounds were quantified (Table 4) by the chromatograms obtained by HPLC. It can be noted that, once the refining phase in casks is completed, the polyphenol content tends to stabilise. While the phenolic acids were fairly stable over time, as in the case of caffeic acid, the anthocyanin content, decreased markedly, especially malvidin, peonidin and petunidin, all of which were present in fairly large amounts at the end of fermentation (fresh wine, sample F). Among the polyphenolic compounds, *t*-resveratrol was present in the samples in quantities ranging from 3 to 5 mg L^{-1}, these values are roughly ten times higher than those reported elsewhere for various samples of Amarone Valpolicella (CELOTTI *et al.*, 1996).

The great amount of data obtained over the four-year period required to produce Amarone wine allowed us to carry out a multivariate statistical analysis to see if there were any correlations between the ageing time of wines and the variables analysed. The PLS statistical method was used to detect the most significant indexes that could explain the

Table 4 - HPLC analysis of Amarone polyphenols (mg L^{-1}). Gallic acid, catechin and epicatechin read at 280 nm; caffeic and *p*-coumaric acids and *t*-resveratrol read at 320 nm; quercetin-3-O-glucoside, quercetin and rutin read at 365 nm (expressed as quercetin); anthocyanins read at 520 nm (expressed as malvidin-3-O-glucoside).

Sample	A Grape juice	B Must (t_0)	C Must (t_{20})	D Must (t_{40})	E Must (t_f),	F Wine, 2001 vintage	G Wine, 2000 vintage	H Wine, 1999 vintage	I Wine, 1998 vintage
gallic acid	1.19±0.39	15.77±1.16	87.06±1.16	154.30±5.15	165.38±3.54	138.16±3.97	119.17±6.42	134.13±5.02	139.13±4.49
catechin	5.57±0.92	10.49±1.09	12.00±0.55	38.71±0.83	29.82±3.14	29.60±2.17	36.45±1.58	24.07±1.14	33.78±2.57
epicatechin	9.49±0.81	9.79±1.07	64.11±0.91	54.19±1.47	65.91±1.11	69.59±5.53	43.76±4.54	53.64±2.56	59.07±1.86
p-coumaric acid	4.80±1.01	6.26±0.75	8.23±1.12	9.03±2.11	7.40±1.32	10.13±0.01	8.19±0.85	13.43±0.24	11.65±0.52
caffeic acid	14.11±0.28	17.87±1.23	16.73±0.08	15.71±0.87	15.52±2.53	35.24±2.15	34.81±1.67	37.96±3.01	35.00±2.05
t-resveratrol	0.69±0.07	1.70±0.28	3.51±1.14	3.88±0.07	3.88±0.07	3.87±0.74	5.30±0.19	3.96±0.01	4.54±0.14
quercetin3-glu	1.35±0.34	2.82±0.36	5.54±0.92	5.89±1.14	7.51±0.99	5.26±1.12	4.73±0.55	6.94±0.83	8.68±1.09
rutin	2.65±0.09	3.39±0.25	4.36±0.84	4.06±0.07	3.66±0.01	4.48±0.77	4.02±0.36	4.30±0.47	3.90±0.43
quercetin	0.11±0.03	2.48±0.74	2.76±0.05	3.37±0.86	3.24±0.34	3.06±0.52	3.18±0.44	2.09±0.99	1.53±0.04
delphynidin	2.14±0.84	3.60±0.81	6.82±0.56	8.77±1.00	8.57±1.05	7.82±1.25	2.68±0.01	4.67±1.05	2.85±0.16
cyanidin	2.30±0.57	4.07±0.24	2.58±0.04	3.12±0.67	3.05±0.07	2.98±0.26	2.66±0.25	2.65±0.71	2.18±0.16
petunidin	2.18±0.24	3.51±1.01	7.44±2.10	10.67±1.05	10.48±2.25	10.20±2.01	2.87±0.08	5.81±1.57	2.84±0.74
peonidin	3.64±0.57	8.54±1.12	7.85±0.94	14.55±0.55	14.51±0.78	14.42±1.09	2.14±0.06	4.10±0.47	2.67±0.01
malvidin	4.85±0.11	11.80±1.58	39.26±3.57	81.67±2.64	78.29±1.69	73.67±2.14	9.58±1.14	17.97±2.93	7.34±1.00

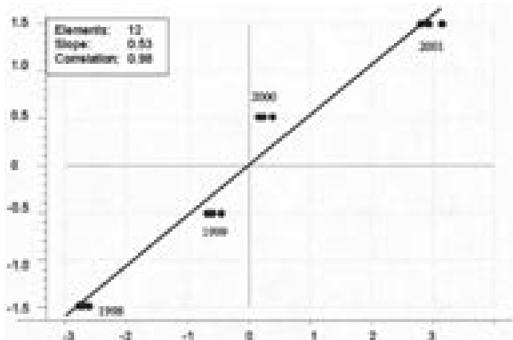

Fig. 2 - Correlation (PLS) between all the chosen variables (X) and the ageing time (Y). For each vintage year, three samples were analyzed.

effect of ageing time on the stability of Amarone wine. The ralues used were normalized to give a single variance to the variables, and thus an equal weight in the analysis. The sample values are scattered along a line and have an excellent multiple correlation coefficient (0.98) (Fig. 2).

From the histograms in Fig. 3 it can be seen that the most significant variables (loading > ± 0.3) along the first principal component are residual sugars, total polyphenols and total anthocyanins, in addition to cyanidin 3-O-glucoside, while pH, gallic and *p*-coumaric acid, catechin and *t*-resveratrol are the most significant variables along the second principal component.

CONCLUSIONS

The consumption of wine, especially red wine, is becoming increasingly more popular not only as a table wine, but also as a cultural, hedonistic event, like tasting a favourite glass of wine with friends and talking about its historical origins. Hence, wine quality must be determined by chemical and physical studies, so that an early evaluation of the shelf-life of basic characteristics may be obtained, in

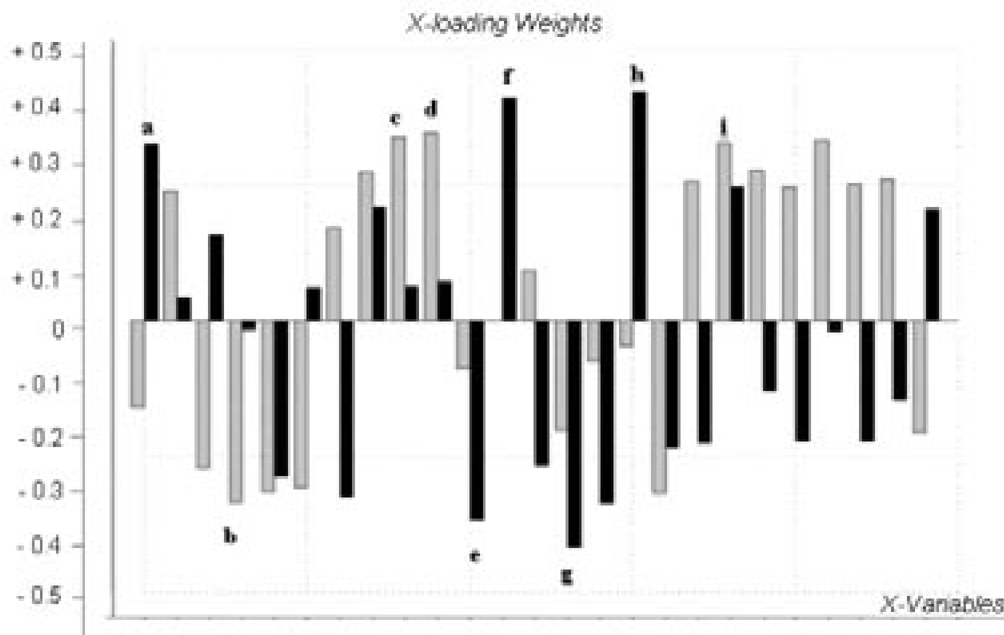

Fig. 3 - Correlation histogram (PLS) of the most significant variables. (loading > ± 0.3). a) pH; b) residual sugar; c) total polyphenols; d) total anthocyanins; e) gallic acid; f) catechin; g) *p*-coumaric acid; h) *t*-resveratrol; i) cyanidin-3-O-glucoside. PC1 grey bars; PC2 black bars. PC (X-expl, Y-expl) 1 (55%, 95%) 2 (31%, 5%).

order to offer consumers a product of unquestionable quality.

Simple analyses and statistics can be used to construct patterns from data collected in the very first years that will serve to predict the ageing and therefore the preservability of a wine.

After estimating the evolution of the chemical and physical parameters in the first four years of life and the ageing time required by law before marketing Amarone Valpolicella, it can be seen that the total polyphenol content is a precise indicator of the evolution of the product over time.

Other parameters, such as pH, residual sugars, colour intensity, total anthocyanins and some polyphenolic monomers (gallic and *p*-coumaric acids, catechin, *t*-resverathrol and cyanidin), are sufficient for determining the ageing possibilities and predicting its shelf life.

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SHORT COMMUNICATION

EFFECT OF DIFFERENT INCUBATION TEMPERATURES ON THE MICROFLORA, CHEMICAL COMPOSITION AND SENSORY CHARACTERISTICS OF BIO-YOGURT

INFLUENZA DI DIFFERENTI TEMPERATURE DI INCUBAZIONE SULLA MICROFLORA, SULLA COMPOSIZIONE CHIMICA E SULLE CARATTERISTICHE SENSORIALI DEL BIO-YOGURT

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ABSTRACT

Yogurt and bio-yogurt were made using a starter culture and a probiotic culture. Incubation was carried out at 37°C and 42°C until pH 4.6 was reached and the yogurts were stored at 4+1°C for 14 days. Yogurts were analysed 1, 7 and 14 days after production. In the samples the levels of acetaldehyde, lactic acid, titratable acidity and whey separation were determined. Viable bacterial counts and sensory assessment were also determined. The incubation temperature and storage time

RIASSUNTO

Una coltura microbica starter e una coltura probiotica sono impiegate per la produzione di yogurt e bio-yogurt. L'incubazione è condotta a 37° e 42°C fino al raggiungimento di valori di pH di 4.6 e i campioni sono conservati per 14 giorni alla temperatura di 4+1°C. Gli yogurt sono sottoposti ad analisi dopo 1, 7 e 14 giorni dalla loro produzione per la determinazione di acetaldeide, acido lattico, acidità titolabile e separazione del siero. Inoltre è stata effettuata la conta dei batteri vivi e l'analisi senso-

- Key words: Fermented milk, lactic bacteria, physical and chemical characteristics, sensory evaluation -

significantly influenced overall properties of the samples. During storage, whey separation, pH and acetaldehyde decreased, but titratable acidity and lactic acid increased. Viable bacterial counts in all bio-yogurts were above 10^7 cfu g^{-1} at the end of storage. Whey separation, titratable acidity and lactic acid contents were lower, while acetaldehyde and viable bacterial counts were higher in the bio-yogurts incubated at 37°C in comparison to bio-yogurt incubated at 42°C. This indicates that lower temperature incubation can be used satisfactorily for the manufacture of bio-yogurt.

riale. La temperatura di incubazione e la durata dello stoccaggio influenzano significativamente tutte le proprietà dei campioni analizzati. Durante la conservazione, si è osservata una diminuzione del valore di pH, di acetaldeide e di separazione del siero, mentre sono aumentati i livelli di acidità titolabile e di acido lattico. In tutti i bio-yogurt la conta dei batteri vivi, effettuata a fine stoccaggio, è risultata essere di circa 10^7 cfu g^{-1}. I bio-yogurt incubati a 37°C mostrano un contenuto minore di acido lattico, acidità titolabile più bassa e minor separazione del siero rispetto a quelli incubati a 42°C, viceversa è risultato più alto il valore dell'acetaldeide e la conta dei batteri vivi. Da quanto esposto, si può dedurre che nella produzione di bio-yogurt si possono impiegare in modo soddisfacente temperature di incubazione più basse.

INTRODUCTION

Since the 1960s, the industrial production of fermented milk, especially yogurt, has increased worldwide. Several factors account for the success of yogurt: its natural appearance, its organoleptic characteristics (fresh and acidulated taste and characteristic flavour), nutritional, prophylactic and therapeutic properties, and its moderate cost (BIROLLO *et al.*, 2000). In recent years, there has been increasing interest in the addition of intestinal bacterial species (*Bifidobacterium* spp. and *Lactobacillus acidophilus* and *Lactobacillus casei*) to fermented milk (VINDEROLA *et al.*, 2000).

A number of factors have been claimed to affect the viability of probiotic cultures in yogurt. Acidity, pH and hydrogen peroxide produced by yogurt bacteria have been identified as having an effect during manufacture and storage. Other factors such as incubation temperature, dissolved oxygen, lactic and acetic acid content in the product, fermentation time, storage temperature, etc. are presumed to affect the viability of probiotic organisms in yogurt (DAVE and SHAH, 1997).

A standard requiring a minimum of 10^6-10^7 colony-forming units per gram (cfu g^{-1}) of *L. acidophilus* and/or *Bifidobacteria* in fermented milk products has been introduced by several food organizations worldwide (IDF, 1992). Consequently, yogurt manufacturers are keenly interested in techniques that can provide reliable counts of probiotic bacteria in their products (TALWALKAR and KAILASAPHATY, 2003). For example, supplementing milk with a combination of casitone, casein hydrolysate, fructose whey protein concentrate, tomato juice and papaya pulp stimulate *L. acidophilus,* while cysteine, acid hydrolysates, tryp-

tone, vitamins, dextrin and maltose improved the viability of bifidobacteria. Prebiotics, such as oligosaccharides are added to food mainly to allow the preferential growth of probiotic organisms. Micro-encapsulation and rupturing yogurt bacterial cells improve the viability of probiotic bacteria (LOURENS-HATTINGH and VILJOEN, 2001). Lower incubation temperatures (37°-40°C) could also be used to improve probiotic organism growth, because it is the optimum temperature range for growing probiotic species (TAMIME and ROBINSON, 1999).

The objective of this study was to determine the effect of incubation temperature on the overall quality and viable bacteria counts in yogurt and bio-yogurt produced from cow's milk.

MATERIALS AND METHODS

Materials

The cow's milk (morning milking) used to manufacture the yogurt and bio-yogurt was collected three times during June 2003 from Holstein cattle in Şanlıurfa in southeastern Turkey. The yogurts were manufactured according to TAMIME and ROBINSON (1999). The milk was inoculated with DVS yogurt culture (FD-DVS-YC-380) consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and probiotic culture (F-DVS yo-Fast 10) consisting of *S. thermophilus*, *L. bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum* BB12 and *Lactobacillus paracasei* subp. *casei* (*L. casei*), obtained from Peyma-Chr. Hansen, Turkey.

Production of yogurt and bio-yogurt

Three different trials were performed for the manufacture of non-stirred plain yogurt and bio-yogurt. In each trial, the large flocks were removed from raw cow's milk using a cloth filter. The fat content of the milk was standardized to 3% by separating the cream.

The milk was heat-treated to 90°C for 10 min, cooled to 45°C and divided into three equal portions (6 L each). The first batch (A) was inoculated with yogurt culture (2% inoculum), and the second and third batches (B and C) were inoculated with probiotic culture (5%) and poured into plastic cups (200 mL). Batches A and B were incubated at 42°C and batch C was incubated at 37°C until pH 4.6 was attained. The yogurts were then transferred to cold storage (4+1°C). According to the Institute of Turkish Standards, the shelf life of yogurt is 14 days. So the yogurts were stored for 14 days and analysed 1, 7 and 14 days after production.

Analytical methods

The pH of the milk, yogurt and bio-yogurt was measured using a digital pH-meter and titratable acidity was determined according to the Soxhlet-Henkel method (YONEY, 1973). The protein, moisture and ash contents of milk, yogurt and bio-yogurt were estimated from the crude nitrogen content of the samples determined by the Kjeldahl, oven-drying and gravimetric methods, respectively (AOAC, 1990). The total fat and lactose contents of milk and yogurt samples were determined by the Gerber (YONEY, 1973) and spectrophotometric (LAWRENCE, 1968) methods, respectively. The lactic acid content of yogurt and bio-yogurt were determined by spectrophotometric methods (STEINSHOLT and CALBERT, 1960). Whey separation of the samples was determined by the method described by KESSLER and KAMMERLAHNER (1982).

Acetaldehyde was quantified by static headspace using Agilent 6890 GC coupled to a headspace sampler according to OTT *et al.* (1999). An aliquot of 5 g of sample was placed into a 10 mL head-

space vial and capped with a teflon seal. The sample was equilibrated at 80°C for 15 min. Headspace (100 µL) was injected onto the GC column using a split ratio of 3. The syringe temperature was kept at 80°C. Volatiles were separated on a DB-Wax column 30 m length, 0.32 mm i.d., 0.5 µm phase thickness. Helium was used as carrier gas at 1 mL min^{-1}. Septum purge was set at 1 mL min^{-1}. The column was kept at 50°C for 6 min, and then the temperature was increased to 80° at 10°C min^{-1} and kept at this temperature for 2 min. The column outlet was connected to an FID detector set at 200°C.

Yogurt and bio-yogurt samples (10 g) were decimally diluted in 100 mL sterile peptone water (0.1%) and 1 mL aliquot dilutions were poured onto plates of the various selective and differential agars in triplicate. M17 agar was used for the enumeration of *S. thermophilus* (RYBKA and KAILASAPHATY, 1996). *L. bulgaricus*, *L. acidophilus*, *B. bifidum* and *L. casei* were incubated by using MRS, MRS with sorbitol agar and MRS-NNLP (DAVE and SHAH, 1996) and MRS-Bile (VINDEROLA and REINHEIMER, 2000), respectively. All plates were incubated at 37°C for 72 h. M17 and MRS-B were incubated aerobically, whereas all other media plates were incubated anaerobically. Anaerobic conditions were created using Anaerocult A sachets (Merck). Plates containing 20-200 colonies were counted and the results are expressed as colony-forming units per gram (cfu g^{-1}) of sample.

The samples were organoleptically assessed by six panelists using a sensory rating scale of 1-10 for flavour and taste, and 1-5 for consistency and appearance as described by BODYFELT *et al.* (1988). The properties evaluated included: (a) six attributes for flavour and taste (no criticism: 10, acid/sour: 9, creamy/milky: 9-7, sweet: 9-7, lack of flavour: 9-7, cooked: 9-6 and other: 5-1), (b) four characteristics of consistency (no criticism: 5, gel-like: 4-2, ropy: 3-1, too firm: 4-2 and too thin: 4-2), (c) four terms describing appearance (no criticism: 5, atypical color: 4-2, lumpy: 4-2, shrunken: 4-2 and syneresis/serum separation: 4-1). The panel of assessors was an external panel of non-smokers who were very familiar with fermented dairy products and were checked on the basis of sensory acuity and consistency.

The experiment was designed according to a 2x3 factorial design. The data were analysed statistically by means of SPSS statistical software program (version 5.0) (DUZGUNES *et al.*, 1987).

RESULTS AND DISCUSSION

The chemical composition of the cow's milk used to produce the yogurt and bio-yogurt (data not shown) fell within the following averages (n=3): titratable acidity 7.31(+0.04) °SH, pH 6.59(+0.02), total solids 12.35(+0.05)%, fat 3.09(+0.2)%, protein 3.64(+0.04)%, lactose 4.76(+0.04)% and ash 0.83(+0.003)%. Some properties of the yogurt and bio-yogurt are shown in Table 1. Different incubation temperatures significantly influenced whey separation, pH, titratable acidity, lactic acid and acetaldehyde content of the bio-yogurt.

Whey separation was greatest in sample A and least in sample C. This could be related to the acidity of the samples. Higher acidity stimulates syneresis in yogurt (TAMIME and ROBINSON, 1999). Syneresis in the samples decreased during storage. This could be due to a decreased net pressure in the protein matrix, which would decrease syneresis (AKIN, 1998). LA TORRE *et al.* (2003) obtained similar results in set-type yogurt and bio-yogurt.

The mean level of acidity of sample A was higher than that of samples B and C. When yogurt cultures are grown with probiotic bacteria, the growth of *L. bulgaricus*, which is the main organism responsible for acid production in yogurt, is inhibited (DAVE and SHAH, 1997). As

Table 1 - Changes in the physical and chemical properties of yogurt and bio-yogurt incubated at different temperatures during storage* (n=3).

Properties	Day of Storage	A	B	C
Whey Separation (mL 25g^{-1})	1	3.57±0.06^{a1}	2.83±0.15^{b1}	2.48±0.12^{c1}
	7	2.84±0.11^{a2}	2.19±0.05^{b2}	1.76±0.12^{c2}
	14	2.22±0.05^{a3}	1.81±0.07^{b3}	1.44±0.04^{c3}
pH	1	4.31±0.,03^{bc1}	4.39±0.03^{b1}	4.44±0.02^{a1}
	7	4.14±0.03^{bc2}	4.20±0.03^{b2}	4.27±0.03^{a2}
	14	4.07±0.01^{bc3}	4.11±0.03^{b3}	4.16±0.03^{a3}
Titratable acidity (°SH)	1	44.0±0.35^{a1}	41.9±0.17^{b1}	40.1±0.36^{c1}
	7	46.2±0.26^{a2}	43.0±0.20^{b2}	42.8±0.26^{b2}
	14	47.9±0.17^{a3}	44.6±0.35^{b3}	44.1±0.10^{c3}
Lactic Acid (mg mL^{-1})	1	0.099±0.001^{a1}	0.092±0.001^{b1}	0.088±0.001^{c1}
	7	0.101±0.000^{a2}	0.094±0.001^{b2}	0.093±0.001^{c2}
	14	0.105±0.001^{a3}	0.100±0.001^{b3}	0.096±0.001^{c3}
Acetaldehyde (ppm)	1	13.66±0.13^{a2}	16.33±0.05^{b2}	17.41±0.12^{c2}
	7	18.07±0.15^{a1}	21.41±0.03^{b1}	22.24±0.13^{c1}
	14	11.14±0.06^{a3}	15.25±0.11^{b3}	15.48±0.10^{c3}

A: Yogurt. B: Bio-yogurt incubated at 42°C. C: Bio-yogurt incubated at 37°C.
* Different letters in the same line indicate significant differences among the samples depending on yogurt type and incubation temperature, and different numbers in the same column indicate significant differences among the samples depending on storage time ($p<0.05$).

a result, the level of acidity in the bio-yogurts was lower than in yogurt. BONCZAR *et al.* (2002) reported similar results. The pH value of sample C was the highest but the titratable acidity and lactic acid contents were lower than those of sample B. Due to the limited growth of *L. bulgaricus* at lower temperatures (KNEIFEL *et al.*, 1993), the acidity level in sample C was lower than in sample B. As expected, storage time significantly affected the acidity level in the samples, titratable acidity and lactic acid content increased, while the pH decreased. DAVE and SHAH (1997), BIROLLO *et al.* (2000), VINDEROLA *et al.* (2000) and BONCZAR *et al.* (2002) reported similar results.

The acetaldehyde content of sample A was lower than in samples B and C. This could be attributed to the level of inoculum and additional lactic bacteria used in those yogurts (*L. acidophilus, L. casei* and *B. bifidum*). It is well-established that *L. bulgaricus* produces more aromatic compounds in milk than *S. thermophilus* (BONCZAR *et al.*, 2002). The activity of alcohol dehydrogenase in a microbial species is important and should not be overlooked. According to FULLER (1989), *L. acidophilus* strains have a lower alcohol dehydrogenase activity than *L. bulgaricus,* which results in less hydrolysis of acetaldehyde to ethanol. Hence, the presence of either lactobacilli species in the starter culture can influence the total acetaldehyde content in these products. The acetaldehyde content of sample C was higher than that of sample B. These results could be attributed to the *L. acidophilus* and *B. bifidum* counts in sample C which were higher than those in sample B. The acetaldehyde content in the samples increased within the first 7 days of storage and then decreased. The

decrease in acetaldehyde content in yogurt and bio-yogurts by the end of the storage period could be due to hydrolysis by microbial enzymes to form other substances such as ethanol (TAMIME and ROBINSON, 1999). Similar results were also reported by BONCZAR *et al* (2002).

Presumptive viable bacterial counts of yogurt samples during storage are shown in Table 2. Presumptive *S. thermophilus* counts were higher in sample C than in the other samples. This could be due to stimulated growth of streptococci species at 37°C. The number of *S. thermophilus* was higher in sample A than in sample B. The *S. thermophilus* counts increased slowly during storage up to day 7, and then decreased by about 1 log cycle. Similar results were reported by BIROLLO *et al.* (2000).

Presumptive *L. bulgaricus* counts in yogurt were slightly higher than bio-yogurts. This could be attributed to the low proportion of *L. bulgaricus* in probiotic culture used in the production, the mechanism of nutritional competition and restrictions in the growth of *L. bulgaricus* due to the presence of probiotic bacteria. *L. bulgaricus* counts in sample B were higher than in sample C. *L. bulgaricus* counts in the samples decreased during storage. Lower storage temperature limits the growth of *L. bulgaricus*, as well as over-acidification (KNEIFEL *et al.*, 1993).

Presumptive *L. acidophilus*, *B. bifidum* and *L. casei* counts in sample C were higher than in sample B. The *L. acidophilus* counts decreased during storage. The most important factors affecting the viability of *L. acidophilus* are acidity and hydrogen peroxide (DAVE and SHAH,

Table 2 - Changes in presumptive viable bacterial counts of yogurt and bio-yogurt incubated at different temperatures during storage* (log cfu g^{-1}) (n=3).

Organism	Day of Storage	A	B	C
S. thermophilus	1	8.92±0.31^{a2}	8.89±0.27^{a2}	9.17±0.28^{b2}
	7	9.51±0.53^{a1}	9.54±0.59^{a1}	9.65±0.37^{a1}
	14	8.65±0.10^{a3}	8.53±0.15^{a2}	8.79±0.05^{a2}
L. bulgaricus	1	8.54±0.06^{a1}	8.39±0.25^{a1}	8.33±0.21^{ab1}
	7	8.06±0.25^{a2}	8.02±0.26^{a2}	7.81±0.12^{b2}
	14	7.56±0.13^{a3}	7.36±0.02^{b3}	7.15±0.07^{bc3}
L. acidophilus	1	-	8.57±0.15^{a1}	8.71±0.14^{a1}
	7	-	7.73±0.35^{a2}	7.85±0.08^{a2}
	14	-	7.66±0.13^{a2}	7.58±0.06^{a3}
B. bifidum	1	-	8.40±0.15^{b1}	8.64±0.15^{a1}
	7	-	7.55±0.02^{b2}	7.75±0.10^{a2}
	14	-	7.23±0.21^{b3}	7.57±0.19^{a2}
L. casei	1	-	8.46±0.12^{b1}	8.74±0.15^{a1}
	7	-	7.95±0.05^{b2}	8.14±0.12^{a2}
	14	-	7.40±0.32^{b3}	7.78±0.13^{a3}

A: Yogurt. B: Bio-yogurt incubated at 42°C. C: Bio-yogurt incubated at 37°C.
* Different letters in the same line indicate significant differences among the samples depending on yogurt type and incubation temperature, and different numbers in the same column indicate significant differences among the samples depending on storage time ($p<0.05$).

Table 3 - Changes in organoleptic properties of yogurt and bio-yogurt incubated at different temperature during storage* (n=3).

Attributes	Day of Storage	A	B	C
Flavour	1	8.07 ± 0.12^{a2}	7.67 ± 0.12^{b3}	7.70 ± 0.17^{b3}
and	7	8.67 ± 0.12^{c1}	9.00 ± 0.00^{b1}	9.20 ± 0.00^{a1}
Taste	14	7.02 ± 0.22^{b3}	8.28 ± 0.10^{a2}	8.27 ± 0.30^{a2}
Consistensy	1	3.67 ± 0.31^{a1}	3.87 ± 0.12^{a2}	3.60 ± 0.20^{b2}
	7	3.93 ± 0.31^{a1}	4.33 ± 0.23^{a1}	4.50 ± 0.10^{ab1}
	14	4.23 ± 0.25^{a1}	4.43 ± 0.06^{a1}	4.43 ± 0.21^{a1}
Appearence	1	3.87 ± 0.12^{ab2}	3.93 ± 0.23^{a2}	3.93 ± 0.12^{a3}
	7	3.93 ± 0.12^{c2}	4.37 ± 0.15^{a1}	4.13 ± 0.12^{b2}
	14	4.33 ± 0.14^{b1}	4.53 ± 0.06^{a1}	4.52 ± 0.28^{a1}
Total Points	1	15.60 ± 0.35^{a2}	15.47 ± 0.46^{a2}	15.23 ± 0.32^{a2}
	7	16.62 ± 0.43^{ab1}	17.70 ± 030^{a1}	17.83 ± 0.21^{a1}
	14	15.75 ± 0.65^{b1}	17.25 ± 0.15^{a1}	17.22 ± 0.70^{a1}

A: Yogurt. B: Bio-yogurt incubated at 42°C. C: Bio-yogurt incubated at 37°C.
* Different letters in the same line indicate significant differences among the samples depending on yogurt type and incubation temperature, and different numbers in the same column indicate significant differences among the samples depending on storage time ($p<0.05$).

1997). Acidity of the samples increased during storage.

Presumptive *B. bifidum* counts decreased during storage which could be attributed to antagonistic relationships between yogurt bacteria and probiotic strains. Despite the higher acid concentration in the samples, the slow decrease in the presumptive *B. bifidum* counts may have been due to the action of *S. thermophilus* as an oxygen scavenger in bio-yogurt and the excellent symbiosis between bifidobacteria and *L. acidophilus* strains. A slow decrease in the number of presumptive *L. casei* cells during storage was observed. This may have been the result of antagonistic relationships between yogurt bacteria and probiotic strains.

The results of the organoleptic evaluation indicated that the bio-yogurts received higher scores than yogurt (Table 3). At the beginning of storage, yogurts were superior to bio-yogurts, mainly because of more intensive flavour and better consistency. However, after 14 days, the yogurt was more acidic than the bio-yogurt, so it received lower organoleptic scores than the bio-yogurts. BONCZAR *et al.* (2002) and LA TORRE *et al.* (2003) also reported that bio-yogurts received higher scores than yogurts. The effects of incubation temperatures on the organoleptic scores of bio-yogurt were negligible. However, sample B received slightly higher scores than sample C. The total scores for the samples increased up to 7 days of storage, and then decreased. This could be associated with the development of acidity and decreases in acetaldehyde contents in the samples. The acetaldehyde content was the highest on day 7, and the lowest on day 14. Acidity also increased during storage. Similar results were found by BONCZAR *et al.* (2002).

CONCLUSION

Incubation temperature had a significant effect on whey separation, lactic acid concentration, acetaldehyde content and viable bacterial counts of bio-yogurt made from cow's milk. During storage, the whey separation and pH decreased, while the titratable acidity and lactic

acid content increased. Viable bacteria counts in all the bio-yogurts were above the threshold of the therapeutic minimum (10^5-10^6 cfu g^{-1}). The viability of the probiotic bacteria was the highest in bio-yogurt incubated at 37°C. The lower acid concentration and higher acetaldehyde content in bio-yogurt incubated at 37°C gave a product that was rated highly by the panelists. Consequently, the lower incubation temperature can be satisfactorily used to produce bio-yogurt.

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SHORT COMMUNICATION

EVALUATION OF THE CHEMICAL QUALITY OF A NEW TYPE OF SMALL-SIZED TOMATO CULTIVAR, THE PLUM TOMATO (*LYCOPERSICON LYCOPERSICUM*)

VALUTAZIONE DELLA QUALITÀ CHIMICA DI UNA NUOVA TIPOLOGIA DI CULTIVAR DI POMODORINO, IL POMODORO DATTERINO (*LYCOPERSICON LYCOPERSICUM*)

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ABSTRACT

The chemical-nutritional attributes were evaluated in plum tomato, a new type of oblong, small-sized tomato that was recently introduced in the European market. Six different cultivars, namely, Dasher, Iride, Navidad, Sabor, 292 and 738, were tested and compared with a cherry-type tomato (cv. Cherubino) which was grown in the same greenhouse under the same conditions. The plum tomato showed important marked differences with respect to the cherry type: more intense

RIASSUNTO

Sono stati valutati gli attributi chimici e nutrizionali di un nuovo tipo di pomodoro di piccola pezzatura, chiamato Datterino, recentemente introdotto nel mercato europeo. Sono state considerate sei differenti cultivar (Dasher, Iride, Navidad, Sabor, 292 e 738) e confrontate con una cultivar di pomodoro ciliegino (cv. Cherubino) coltivata nella stessa serra e nelle medesime condizioni. I risultati hanno mostrato alcune differenze significative rispetto alla tipologia ciliegino: un più intenso colore

- Key words: ascorbic acid, carotenoids, cherry tomato, phenolics, plum tomato -

red colour, lower acid content and a higher lycopene content. The quality attribute values for cv. 738 were higher than the average values for the other cultivars. In contrast, the cherry-type variety had slightly higher levels of ascorbic acid and β-carotene than the plum tomatoes.

rosso, una minore quantità di acidi ed un maggiore contenuto di licopene. Gli attributi di qualità della cv. 738 sono stati superiori ai valori medi. Il ciliegino, invece, ha mostrato quantità leggermente più elevate di acido ascorbico e di β-carotene.

INTRODUCTION

The nutritional value of tomato is due to the beneficial effects of some health-promoting constituents, (vitamins, fibre and carotenoids) which in general, help inhibit oxidative processes and in particular, help prevent some types of cancer and cardiovascular diseases, (PARFITT *et al.*, 1994; GIOVANNUCCI, 1999; LAVELLI *et al.*, 1999, 2000; RAO and AGARWAL, 2000).

It is well established that small-sized tomatoes are usually characterized by higher levels of dry matter and soluble solids than normal-sized tomatoes; these differences are due to higher content of sugars and organic acids, which, in turn, are the major factors in determining the greater sweetness, sourness and overall flavor intensity (PICHA, 1986; LEONARDI *et al.*, 2000; PAGLIARINI *et al.*, 2001).

A new hybrid of small-sized tomato called "plum tomato" has recently been introduced on the market and has aroused great consumer interest. As indicated by the breeders, the plum tomato variety originated from inter-specific crossbreeding among *Lycopersicon lycopersicum*, *Lycopersicon pimpinellifolium* (Red Currant-type) and *Lycopersicon chesmanii* (PASSERI, 2003). The shape of a plum tomato is similar to a small plum or date, hence the Italian name "Datterino". The small size (10-15 g) and very pleasant taste make it a valuable and special product. Due to these characteristics, demand for plum tomato has increased rapidly in the last few years.

To date, no study has been performed on plum tomatoes, apart from a recent study on the effect of packaging on their shelf life (MURATORE *et al.*, 2005). In the present paper, the level of the most important constituents (sugars, acids, phenolics and carotenes) of six different plum tomato cultivars was assessed and compared with the results of a cherry-type tomato which was chosen as a standard because of its small size and high quality. The quantitative distribution of sugars, ascorbic acid, phenolics and carotenes in cherry tomatoes is not only dependent on the cultivar (HART and SCOTT, 1995; ABUSHITA *et al.*, 2000; LEONARDI *et al.*, 2000; ARENA *et al.*, 2003), but also on agronomic factors (LA MALFA *et al.*, 1995; DE PASCALE *et al.*, 2001), ripening stage and post-harvest storage (GIOVANELLI *et al.*, 1999; ARIAS *et al.*, 2000; RAFFO *et al.*, 2002). In order to minimize the effects that were not due to the cultivar, it was compared directly with the cherry-type tomato. The cherry-type tomato was transplanted and grown in the same greenhouse, under the same agronomic conditions, and its samples were harvested at the same commercial ripening stage, following the indications of the producer.

MATERIALS AND METHODS

Sampling and sample preparation

The following six plum tomato cultivars were tested: Dasher, Iride, Navidad, Sabor, 292 and 738; the cherry-type variety Cherubino was used as a reference. Each sample of the tomato varieties consisted of about 50 fruits (800-1,000 g) collected from different plants. Nine different samples of each plum and cherry tomato variety were harvested at the commercial ripening stage, from December 2002 to April 2003 (the sampling dates were: Dec. 6 and 18 2002; Jan. 7, 15 and 28 2003; Feb. 12 2003; Mar. 4 and 13 2003; Apr. 5 2003). Overall, 63 samples were examined.

Carpometric and colourimetric measurements were taken and the pH, acidity, soluble solids and dry matter values were determined on fresh tomatoes, while reducing sugars, phenolic compounds, ascorbic acid and carotenoids were determined just after thawing tomato samples which had been stored at -18°C.

Physical measurements

The length, width and weight were measured on five fruits randomly chosen from each sample; the mean values and standard deviations were then calculated. The CIE chromatic coordinates L*, a* and b* were also determined on the same five fruits by testing four different points on the tomato surface, using a portable colorimeter mod. NR-3000 (Nippon Denshoku Ind. Co. Ltd.).

Chemical determinations

All chemical determinations were carried out on two lots of fifteen randomly selected fruits. Each analysis was performed in duplicate.

The pH was measured on homogenized samples using a Mettler Toledo MP220 pH-meter, previously calibrated with buffer solutions. The acid contents were determined by titration with 0.1 N NaOH and are expressed as mg of monohydrate citric acid per 100 g of fresh tomato. Soluble solids were measured on homogenized and filtered samples with a refractometer (Zeiss, mod. 16531) and are expressed as degrees Brix at 20°C. Dry matter was determined using about 2 g of homogenized sample after drying in a ventilated oven at 70°C until constant weight was reached.

Ascorbic acid was extracted from the sample as described by NISPEROS-CARRIEDO *et al.* (1992), and quantified by HPLC by calibration with solutions of a pure standard (Extrasynthése, Genay, France).

Total phenolics were extracted by rinsing the homogenized samples (5 g) with warm water, filtering on paper, and collecting the extract in a 250 mL volumetric flask. A four-fold diluted sample was analyzed using the Folin-Ciocalteau method (SINGLETON and ROSSI, 1965), and results are expressed as mg of gallic acid per 100 g of fresh product.

Glucose and fructose were extracted from homogenized tomato samples (10 g) with warm water, filtering on paper and collecting the extract in a 250 mL volumetric flask; the extract was then diluted five-fold. Determination of each reducing sugar was performed by an enzymatic-spectrophotometric method using the test combination kit (Boehringer Mannheim, Germany).

Carotenoids were extracted and separated by HPLC using the procedure of DE SIO *et al.* (1999). Detection was performed at 450 nm for lutein and β-carotene, at 470 nm for lycopene, at 285 and 350 nm for phytoene and phytophluene, respectively. Quantification was made with the external standard method using pure β-carotene, lutein (Extrasynthése, Genay, France) and lycopene (Sigma-Aldrich, Milan, Italy).

Statistical analysis

Experimental data were elaborated by ANOVA, using the Statgraphic Plus software (Manugistic Inc. Rockville, MD, USA). The method used to discriminate among the means (Multiple Range Test) was Fisher's Least Significant Difference (LSD) procedure at 95.0% confidence level.

RESULTS AND DISCUSSION

Physical characterization

The cultivars of plum and cherry tomatoes were preliminarily characterized according to weight, dimension, shape and colour (Table 1).

The Iride, Sabor and 738 cultivars had a higher length/width ratio due to their long, narrow shape. Sabor is bigger and heavier than the other cultivars. The average weight of a plum tomato, excluding Sabor, is 13.5 g, while that of the cherry-type variety is 18.8 g. Plum- type tomatoes are smaller, and this attribute is appreciated by consumers from a merely aesthetic point of view.

The colour parameters L* and b* showed no significant variation among the cultivars, whereas the red parameter a* changed from 8.8 for cherry to 12.2 for Navidad. Plum tomato cultivars were characterized by a more intense red hue than the cherry-type, as indicated by the higher $(a^*/b^*)^2$ ratio, which is an index of red-colour development (ARIAS *et al.*, 2000).

Chemical characterization

Table 2 reports the chemical characteristics of each cultivar. The pH and acidity values were similar for all the plum tomatoes, but the cherry-type tomato was the most acidic. Sabor had the lowest soluble solids and dry matter values, while 738 had the highest values (9.1 and 11.1 mg/100 g, respectively). The cherry-type tomato had mean values that are very similar to most of those of the plum tomatoes, but the values were lower than those for cultivar 738.

The glucose and fructose levels were always high, with fructose being slightly higher (~7%). Cultivar 738 had the highest total sugar content, which corresponds to having the highest dry matter and soluble solids, of which sugars made up about 55% and 67%, respectively. Reducing sugars amounted to 6.13 g/100 g for cv. 738, and the mean value for the plum-type cultivars, excluding Sabor, was 5.70 g/100 g. This is a very

Table 1 - Carpometric and colourimetric characteristics of plum and cherry tomato cultivars.

Variable	Dasher	Iride	Navidad	Sabor	292	738	Cherry
Weight (g)	16.1^{bc}	9.7^{a}	11.6^{a}	26.3^{e}	16.2^{c}	13.9^{b}	18.8^{d}
Length (cm)	3.5^{c}	3.2^{a}	3.2^{b}	4.5^{d}	3.5^{c}	3.5^{c}	2.9^{b}
Width (cm)	2.7^{c}	2.2^{a}	2.4^{b}	3.1^{d}	2.8^{c}	2.5^{b}	3.2^{d}
L*	24.7^{ab}	24.5^{a}	26.1^{b}	26.1^{b}	24.9^{ab}	23.9^{a}	24.1^{a}
a*	10.0^{bc}	10.0^{b}	12.2^{e}	11.1^{d}	10.1^{bcd}	11.0^{cd}	8.8^{a}
b*	20.6^{ab}	20.8^{ab}	21.5^{b}	21.0^{ab}	20.1^{ab}	19.6^{a}	19.4^{a}
$(a^*/b^*)^2$	0.24^{ab}	0.23^{ab}	0.32^{d}	0.28^{bcd}	0.25^{abc}	0.31^{cd}	0.21^{a}

(a) Each value is the average of nine samples. Means in the same row followed by a common letter are not significantly different (P<0.05).

high sugar concentration in comparison with the values reported by PICHA *et al.* (1986), PAGLIARINI *et al.* (2001) and RAFFO *et al.* (2002) in some cherry-type tomato cultivars. The sugars/acidity ratio value in the cherry-like Cherubino was low because it had the highest acidity, while Sabor had the lowest ratio because it had the lowest concentrations of sugars.

The mean ascorbic acid content was slightly higher in the cherry-like Cherubino (31.3 mg/100 g) than Dasher (28.5 mg/100 g), Navidad (25.0 mg/100 g), 292 (26.1 mg/100 g) and 738 (25.1 mg/100 g), whereas it was significantly higher than Iride and Sabor. These values are comparable with those reported in the literature (RAFFO *et al.*, 2002; ARENA *et al.*, 2003). However, the mean value for the cherry-type, was characterized by a high standard deviation, and was not statistically different from Dasher and 292.

The phenol compounds in fruits and vegetables are of special importance from a nutritional point of view, especially for their antioxidant capacity. The phenolic fraction of tomato includes different flavonoid glycosides and esters of hydroxycinnamic acids, prevalently represented by the derivatives of naringenin, quercetin and caffeic acid, characterized by high antioxidant activity (HOLLMAN *et al.*, 1996; CROZIER *et al.*, 1997; STEWART *et al.*, 2000; RAFFO *et al.*, 2002). The level of phenolic substances in plum tomatoes was very high, ranging from 42.5 mg/100 g for cv. Sabor to 74.9 mg/100 g for cv. 738, while the cherry-type Cherubino averaged 63.9 mg/100 g, which was higher than that observed in the cherry tomato cv. Naomi (ARENA *et al.*, 2003). It has been noted that cherry tomatoes grown in warm, sunny countries are richer in polyphenols, particularly in quercetin derivatives (STEWART *et al.*, 1997). Moreover, since polyphenols are located prevalently in the pericarp of the fruit, the level of these compounds in small-sized tomatoes is higher than in normal-sized ones because of the greater skin/volume ratio (STEWART *et al.*, 1997).

The distribution trends of the major carotenoids differed between the plum tomato cultivars and the cherry-like variety. The mean lycopene content in the plum tomato cultivars was higher than that in the cherry-like variety (4.65 and 3.43 mg/100 g, respectively). The highest concentration was estimated in Sa-

Table 2 - Chemical characteristics of plum and cherry tomato cultivars.

Variable	Dasher	Iride	Navidad	Sabor	292	738	Cherry
pH	4.09^{ab}	4.13^{a}	4.12^{a}	4.21^{b}	4.10^{ab}	4.09^{ab}	4.05^{a}
Acids (g/100 g)	0.78^{cd}	0.73^{bc}	0.67^{ab}	0.64^{a}	0.74^{abc}	0.72^{bc}	0.85^{d}
°Brix	8.8^{b}	8.8^{b}	8.1^{b}	6.5^{a}	8.7^{b}	9.1^{b}	8.8^{b}
Dry matter (g/100 g)	10.8^{bc}	10.4^{bc}	9.7^{ab}	8.6^{a}	10.6^{bc}	11.1^{c}	10.8^{bc}
Glucose (g/100 g)	2.78^{bc}	2.71^{bc}	2.48^{b}	2.02^{a}	2.85^{bc}	2.99^{c}	2.86^{bc}
Fructose (g/100 g)	2.85^{b}	2.92^{b}	2.81^{b}	2.22^{a}	2.96^{b}	3.14^{b}	3.16^{b}
Total sugars/acids	7.26^{ab}	7.65^{abc}	7.96^{bc}	6.70^{a}	7.86^{bc}	8.53^{c}	7.20^{ab}
Ascorbic acid (mg/100 g)	28.5^{c}	18.1^{ab}	25.0^{bc}	13.4^{a}	26.1^{c}	25.2^{bc}	31.3^{c}
Phenol compounds (mg/100 g)	68.8^{bc}	69.7^{bc}	61.2^{b}	42.5^{a}	67.5^{bc}	74.9^{c}	63.9^{bc}
Lycopene (mg/100 g)	3.98^{ab}	4.45^{bc}	4.89^{bc}	5.22^{c}	4.57^{bc}	4.77^{bc}	3.43^{a}
β-Carotene (mg/100 g)	0.68^{ab}	0.80^{cd}	0.89^{de}	0.67^{a}	0.78^{bc}	0.80^{cd}	0.99^{e}

Each value is the average of nine samples separated into two lots; each lot was analyzed in duplicate. Means in the same row followed by a common letter are not significantly different ($P<0.05$).

bor, followed by Navidad and 738 (Table 2). In contrast, the cherry-like variety had higher β-carotene (0.99 mg/100g) than that observed in the plum-types (0.77 mg/100 g). Lycopene made up about 85% of the total carotenoids in the plum cultivars, except Sabor (88.6%), and 77.6% in the cherry type Cherubino. Conversely, β-carotene made up about 15% and 22.4% in plum and cherry-type tomatoes, respectively. Lutein was present at a concentration of about 0.1 mg/100 g, while traces of the colourless carotenoids (phytoene and phytophluene) were detected.

The plum tomato cultivars examined had high levels of sugars and health-promoting components (ascorbic acid, phenolic compounds and carotenoids). They also had an intense red colour and high sugars/acidity ratio. The quality parameters are compared in Table 3, where the + and – symbols indicate the highest and lowest mean values of each parameter for each plum and cherry tomato cultivar. Sabor is the least valuable cultivar, despite having had the highest lycopene content; while 738 is the most valuable having had the maximum values for most of the parameters. The cherry tomato Cherubino had the highest ascorbic acid and β-carotene contents, while cultivars Dasher and 292 were characterized by a balanced content of all the components. Finally, Navidad had the most intense red colour, and Iride is the most appealing because of its shape and being the smallest of the cultivars examined.

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Table 3 - Comparison of the characteristics of plum and cherry tomato cultivars.

Parameter	Navidad	Sabor	738	Cherry Cherubino
$(a^*/b^*)^2$	+			–
Solubile solids		–	+	
Dry matter		–	+	
Sugars/acidity ratio		–	+	
Ascorbic acid		–		+
Phenol compounds		–	+	
Lycopene		+		–
β-Carotene		–		+

The + or – signs denote the cultivar showing the highest or lowest mean value for each parameter, respectively. The cultivars Dasher, Iride and 292 are not reported because they showed intermediate values.

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GC/MS-SIM ANALYSIS OF PHENOLIC COMPOUNDS IN OLIVE OIL WASTE WATERS

ANALISI DI COMPOSTI FENOLICI IN ACQUE DI VEGETAZIONE DA FRANTOIO OLEARIO CON TECNICA GC/MS-SIM

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ABSTRACT

Simple phenolic compounds in olive oil waste waters have been determined by gas-chromatography-electron impact mass spectroscopy-selected ion monitoring mode (GC/MS-SIM). The phenolic compounds extracted from waste waters were derivatized by treatment with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and fourteen phenolic compounds were identified. Preparation of calibration standards and curves are described. The recoveries for all the phenols examined

RIASSUNTO

Sono stati analizzati alcuni composti fenolici a struttura chimica semplice presenti in acque di vegetazione da frantoio oleario avvalendosi della tecnica GC/MS-SIM. I composti fenolici estratti dalla matrice sono stati derivatizzati con BSTFA e sono stati identificati e quantificati quattordici composti fenolici. I recuperi di tutti i composti esaminati rientravano in un ampio range di valori: 49-100%. Il metodo analitico utilizzato permette di determinare tutti i composti fenolici anche

- Key words: antioxidants, GC-MS, olive oil waste waters, phenols -

were in a wide range (49-100%). This analytical method also allowed the minor components of the phenolic mixture extracted from waste waters to be determined. The detection limit varied from 0.5 mg/L for tyrosol to 1.3 mg/L for sinapic acid.

quelli presenti in concentrazioni molto basse, inoltre il limite di sensibilità variava da 0,5 mg/L per il tirosolo a 1,3 mg/L per l'acido sinapico.

INTRODUCTION

During the past few decades, the role of phenolic compounds as antioxidants and free radical scavengers (BRIANTE *et al.*, 2002; VISIOLI *et al.*, 1999) has attracted considerable public and scientific interest. The classical method for determination of phenols is the Folin-Ciocalteu procedure (CATALANO *et al.*, 1999). This is a colorimetric method that evaluates the total concentration of phenols but can not be used as a tool to identify and quantify individual phenolic compounds; furthermore, since other compounds present in the matrix may contribute to the absorbance, this method is characterized by poor specificity.

Single phenolic derivatives in olive oil waste waters have been determined mainly by Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC). The latter has often been preferred because it does not require any kind of derivatization of the samples prior to analysis (MONTEDORO *et al.*, 1992; ANGEROSA *et al.*, 1995; PEREDES *et al.*, 1999; MULINACCI *et al.*, 2001; CECCON *et al.*, 2001; ZHANG and ZUO, 2004). However since the HPLC method generally uses UV detection, the predominant phenols such as tyrosol and hydroxytyrosol can be satisfactorily analyzed but the minor components (CECCON *et al.*, 2001) can not be.

In this paper the application of gas chromatography-electron-impact mass spectroscopy-selected ion monitoring (GC/MS-SIM) to the analysis of phenolic derivatives in olive oil waste waters is reported. This method is known to be an innovative analytical technique with great potential (KIM *et al.*, 2003; SHIN *et al.*, 2003). Although it requires derivatization of the phenolic and benzoic compounds with BSTFA, it has high sensitivity, good repeatibility and easy application. Moreover the method allows satisfactory determination of both the predominant and minor components of the phenolic mixtures.

MATERIALS AND METHODS

Chemicals

m-Methoxy-acetophenone (internal standard - IS), syringic acid, caffeic acid, 3,4-dihydroxy-phenylacetic acid, p-coumaric acid, ferulic acid, trans-cinnamic acid, tyrosol, vanillin, homogentisic acid, homovanillic acid, veratric acid, vanillic acid, sinapic acid and N,O-bis (trimethyl silyl)trifluoroacetamide (BSTFA) used for this study were purchased from Aldrich Chemical Co (Milano, Italia).

Hydroxytyrosol was prepared according to a previous procedure (CAPASSO *et al.*, 1999).

Waste waters (WW) from an olive oil production plant in Abruzzo were used.

Sample preparation for GC/MS-SIM

WW (200 mL) was acidified (pH = 2) with a 37% aqueous hydrochloric acid

solution and then submitted to continuous liquid-liquid extraction with ethylacetate (500 mL) for 10 h. The extract was dried (Na_2SO_4) and evaporated under reduced pressure and at 30°C to afford a residue (2.18 g) which was derivatized.

An aliquot of the residue (18.48 mg) was weighed in a 10 mL volumetric flask and a large excess of BSTFA (1 mL) and acetone (5 mL) were added. The mixture was stirred at 30°C for 3 h, then diluted to the mark with acetone to obtain the concentration of 1.848 mg/mL.

In order to carry out the GC-MS analysis, 0.3 mL of an acetone solution of m-methoxy-acetophenone (IS) (conc. 1.895 mg/mL) was added to the acetone solution of the derivatized sample (0.7 mL). The concentration of the resulting solution was 1.294 mg/mL ($Conc_{IS}$ = 0.5685 mg/mL). A 1.0 µL aliquot of the solution was analyzed by GC-MS.

Preparation of calibration standards and curves

A stock standard solution was prepared by dissolving about 40 mg each of 1-14 standard compounds in 10 mL of acetone in a 20 mL volumetric flask. The solution was then derivatized by treatment with 1.0 mL of BSTFA for 3h at 30°C; after that, acetone was added to the mark. The working standard solutions were prepared by combining separately 10, 25, 50, 75, and 100 µL each of the stock standard solution with 0.3 mL of the IS solution (conc. 1.895 mg/mL) in a 1 mL volumetric flask. The resulting solutions were diluted to the mark with acetone to obtain the calibration solutions in a range of concentrations of 20-200 mg/L ($Conc_{IS}$. 568.5 mg/L). Calibration curves were constructed by a linear regression of the peak area ratio of the individual phenolic standard to the m-methoxyacetophenone as internal standard (IS), versus the molar ratios of the IS and each phenol on each analysis of the standard solutions: the correlation coefficients were $r^2 \geq 0.993$.

Instrumentation

Capillary GC-MS analysis was carried out on a Hewlett Packard 5970 system (Palo Alto, CA 94303,USA).

GC was performed on 5% PHME – Siloxano fused-silica capillary column (30x0.25 mm I.D.) with the stationary phase coated at a 0.25 µm film thickness. Helium was used as the carrier gas at a column head pressure of 7 psi. The initial column temperature was set at 70°C. After sample injection, the temperature was maintained at 70°C for 3 min, then increased at 8°C/min to 320°C and maintained at this temperature for 15 min.

The temperature of the injector was 260°C. The mass spectrometer was operated in the electron impact mode at 70 eV.

RESULTS AND CONCLUSIONS

The chemical structures of the 14 standard compounds studied are reported in Fig. 1.

The phenolic compounds of both standards and the extraction mixture were derivatized by treatment with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (ANGEROSA *et al.*, 1995). The best experimental conditions that emerged from several assays are described in the Materials and Methods Section. The complete conversion of the phenols into the corresponding silylated products was confirmed by monitoring the reaction progress by TLC.

A GC - chromatogram of the silylated polyphenols in a standard solution is reported in Fig. 2 which clearly shows that all compounds were well resolved from each other in less than 35 min.

Table 1 reports the retention times and characteristic ions present in the

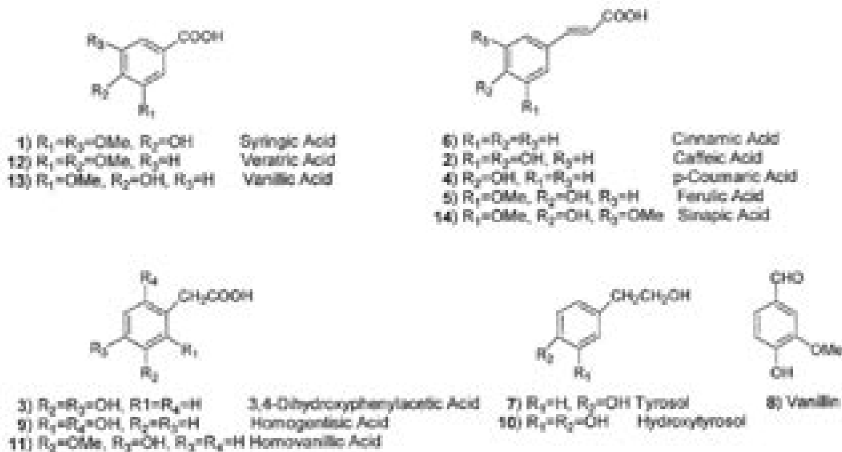

Fig. 1 - Structures of phenolic compounds 1-14.

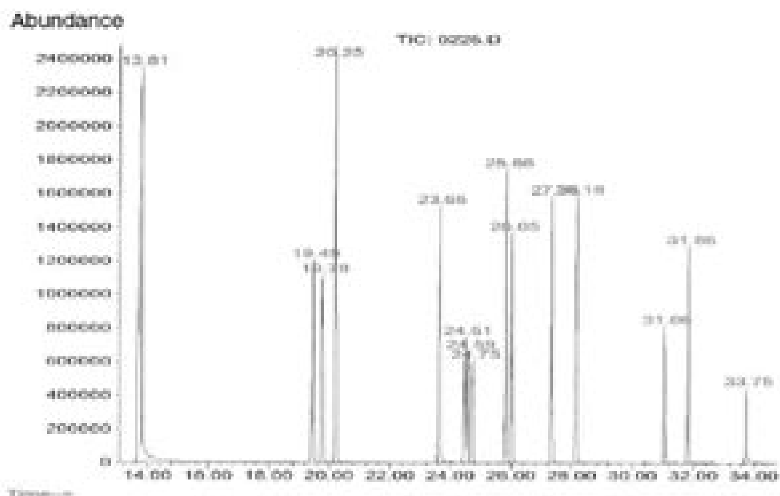

Fig. 2 - GC/MS-SIM chromatogram of standard phenolic compounds (100 μg/mL).

MS spectra together with the target and qualifying ions by GC/MS-SIM of the fourteen standard phenolic compounds and internal standard used.

The concentration of the compounds identified in WW was determined by relating their peak areas to that of IS. The calibration curves were linear for all phenolic compounds with correlation coefficients $r^2 \geq 0.993$.

The entire analytical procedure described under Sample Preparation for GC/MS-SIM (in Materials and Methods) was applied to the WW sample, which was spiked with increasing amounts of the standard solution of the fourteen phenols (1, 5, 10 mL), containing about 10 μg/mL of each component. Recoveries (Rec) were determined as usual from the ratio of the amount found (C_F) to the sum of the amount added (C_{Ad}) plus that originally present in the matrix (C_O).

$$Rec = C_F/C_{Ad}+C_O$$

Table 1 - GC/MS-SIM parameters of silylated derivatives of phenols 1-14 and IS in the standard solutions.

Standard	Retention time min.	Identified ions m/z (rel. intens.)	Qualifier ions m/z
IS	13.61	150[a] (M^+, 68), 135 (100), 107 (48), 92 (24), 77 (39)	135, 107
8	19.49	224[a] (M^+, 28), 209 (43), 194 (100), 73 (27), 59 (11)	209, 194
6	19.78	220[a] (M^+, 29), 205 (100), 161 (57), 131 (87), 103 (67)	205, 161
7	20.25	282[a] (M^+, 22), 2675 (16), 193 (14), 179 (100), 73 (45)	267, 179
12	23.65	254[a] (M^+, 58), 239 (85), 195 (100), 165 (86), 79 (13)	239, 195
10	24.51	370[a] (M^+, 44), 267 (100), 193 (23), 179 (13), 73 (64)	267, 193
13	24.59	312[a] (M^+, 65), 297 (100), 282 (34), 267 (71), 253 (48), 223 (50)	297, 253
11	24.75	326[a] (M^+, 73), 311 (44), 267 (38), 209 (67), 179 (39), 73 (100)	267, 179
3	25.86	384[a] (M^+, 61), 267 (48), 237 (15), 179 (51), 73 (100)	267, 179
9	26.05	384[a] (M^+, 50), 341 (27), 252 (15), 147 (19), 73 (100)	341, 252
1	27.36	342[a] (M^+, 74), 327 (100), 312 (72), 297 (61), 253 (42), 73 (92)	327, 312
4	28.18	308[a] (M^+, 80), 293 (100), 249 (50), 219 (84), 73 (99)	293, 249
5	31.06	338[a] (M^+, 100), 323 (56), 308 (51), 249 (45), 73 (94)	323, 308
2	31.86	396[a] (M^+, 87), 381 (22), 219 (84), 191 (16), 73 (100)	381, 219
14	33.86	368[a] (M^+, 100), 253 (47), 338 (91), 279 (19), 73 (40)	353, 338

[a]Target ion.

Since in the above equation Rec and C_O were unknown, a linear relationship (C_F = Rec x C_{Ad} + I) for each of the fourteen phenols was established, and the values for slope and intercept were the mean Rec and I = Rec x C_O, respectively ($r^2 \geq 0.988$).

As reported in Table 2, recoveries (Rec) for all the phenols examined were in a wide range (49-100%). The detection limit varied from 0.5 mg/L for tyrosol to 1.3 mg/L for sinapic acid. Due to the wide range of polarity and high water solubility of the polyphenols, the efficiency of the ethylacetate continuous liquid-liquid extraction seems to be good.

The results of the analysis of the extract from WW are also reported in Table 2; the concentrations are expressed as amount (mg) of each phenol detected in the extract obtained from 100 mL of WW. Although the GC-MS/SIM technique requires the extraction-derivatization procedure, this method allowed the phenols present in the sample to be qualitatively and quantitatively determined also at very low concentrations. HPLC allowed aqueous matrixes to be directly analysed but it was less sensitive, and as previously reported (CECCON *et al.*, 2001), only tyrosol (7), hydroxytyrosol (10) and caffeic acid (2) were detected in WW by this method. Tyrosol (7) and hydroxytyrosol (10) were the predominant products and their concentrations are in agreement with those previously determined by the HPLC method.

In conclusion GC/MS-SIM has been shown to be a simple and sensitive analytical tool for the determination of phenolic compounds in olive oil waste wa-

Table 2 - Extraction recovery and concentration of identified phenolic compounds in the olive oil waste waters (WW).

Compound	Rec x 100[a]	Concn in WW (mg/100 mL)
1) Syringic acid	65.1±4.8	0.3
2) Caffeic acid	60.0±4.3	8.1
3) 3,4-dihydroxy-phenylacetic acid	63.2±3.9	n.d.[b]
4) p-Coumaric acid	65.4±5.8	2.4
5) Ferulic acid	66.4±5.1	0.4
6) trans-Cinnamic acid	58.9±3.8	1.1
7) Tyrosol	55.9±4.5	24.7
8) Vanillin	66.7±4.1	n.d.
9) Homogentisic acid	82.7±5.2	n.d.
10) Hydroxytyrosol	74.4±5.5	72.7
11) Homovanillic acid	57.6±4.1	0.1
12) Veratric acid	69.6±4.7	0.8
13) Vanillic acid	49.2±6.3	7.3
14) Sinapic acid	100.0±7.1	n.d.

[a] Recovery and standard deviation of three replicates.
[b] Not detected.

ter. The application of this method for the detection of polyphenols in different matrixes is in progress.

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COMPARISON OF EC BROTH AND 2% BRILLIANT GREEN BILE BROTH

COMPARAZIONE TRA EC BROTH E BRILLIANT GREEN BILE BROTH AL 2%

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ABSTRACT

The efficacy of two culture media, EC broth and 2% Brilliant Green Bile broth, were compared for the determination of total and fecal coliforms in foods. The total coliform results were: superposition (26.92%), greater specificity of EC (46.16%), greater specificity of 2% Brilliant Green Bile broth (26.92%). Regarding fecal coliforms, results were: superposition (57.69%), greater specificity of EC (28.85%), greater specificity of 2% Brilliant Green Bile broth (13.46%). The results con-

RIASSUNTO

Lo scopo del presente studio è quello di comparare l'efficienza di due terreni di coltura usati per la determinazione di coliformi totali e fecali in prodotti alimentari: EC broth e Brilliant Green Bile broth 2% (BBVB). Per quanto riguarda i coliformi totali i risultati delle analisi nei due terreni sono stati: sovrapposizione nel 26.92% dei casi, maggiore specificità dell'EC nel 46.16% contro il 26.92% del BBVB al 2%. Nel caso dei coliformi fecali vi è stata una sovrapposizione dei risultati nel 57.69%, una

- Key words: coliforms, culture media, food -

firm the greater specificity of EC broth for the determination of total and fecal coliforms in food.

maggiore risposta dell'EC nel 28.85%, mentre del 13.46% del BBVB. I risultati tendevano a confermare una maggiore specificità di EC broth per la determinazione di coliformi totali e fecali in prodotti alimentari.

INTRODUCTION

Coliforms, a group of species from several different genera, are generally grouped together because of their common characteristics. Total and fecal coliforms are Gram-negative, facultative anaerobes. Many are motile, nonsporeforming rods and ferment lactose, producing acid and gas within 48 hours at 35°, 37° or 44°C. They are able to grow in food except for food that has a pH of 4.0 or less and an Aw of 0.92 or less. Some fecal coliforms can be present in raw food of animal origin or from plants grown on contaminated soil and water. However their presence, especially above a certain level, is viewed cautiously for fecal contamination and the presence of enteric pathogens.

The Department of Hygiene of the University of Messina has extensively used fecal coliforms as indicators of potential risk in food. The question about specificity and sensitivity of culture media for determination of total and fecal coliforms in food has been discussed by many authors (ACEVADO *et al.*, 2001; CAKIR *et al.*, 2001; SUWANSONTHICAI and RENGPIPAT, 2003; HAWEMEISTER *et al.* 1991; CLARK *et al.*, 1993; GEISSLER *et al.*, 2000; PARK *et al.*, 2001; WARBUTON, 2000; WEAGANT and FENG, 2001). In particular, LECLARQUE *et al.* (2002) compared a 24h direct plating method for enumeration of fecal coliforms using fecal coliform agar (FCA), with the 24h standardized violet red bile lactose agar (VRBL) method. MULLER *et al.* (1990) studied the determination of total and fecal coliforms for quality control of bathing water using the EC guideline 76/160 most probable number and Brila-Mug broth. CATTAI *et al.* (2000) compared traditional and chromogenic media for determination of coliforms and *E. coli* in water.

2% Brilliant Green Bile broth is a selective medium for the confirmation of the presence of coliform bacteria in water (APHA, 1980), dairy (APHA, 1978) and other food products (APHA, 1976). It is widely used particularly in food microbiology laboratories outside Europe. In European countries, lauryl tryptose broth with tryptophane is the preferred media. EC broth is a lactose buffered broth with bile salts recommended by the American Public Health Association (APHA, 1985) for the detection of fecal coliforms in water and food.

This study was undertaken to compare 2% Brilliant Green Bile broth, usually used by our group for the determination of total and fecal coliforms in food, and EC broth, which has not been previously tested, but whose efficiency needs to be evaluated.

MATERIALS AND METHODS

Fifty-two food samples were examined that were comprised of twenty mozzarella cheese, twenty vegetable samples and twelve meat samples. All the samples were raw and twenty-five grams per sample were diluted with 225 mL of 0.9% sa-

line solution, 0.1% peptone. The samples were homogenized in a stomacher and afterwards 10 fold successive dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) were made. One milliliter of each dilution was used to inoculate 3 tubes of 2% Brilliant Green Bile broth and 3 tubes of EC broth. EC broth was prepared according the formulation reported by the International Standardization Organization (ISO) 7251 and 2% Brilliant Green Bile broth according the formulation reported by the International Standardization Organization (ISO) 4831.

Microbial growth and gas production at 35±2°C after 24-48 h demonstrated the presence of total coliforms, while microbial growth and gas production at 44°C after 24-48 h demonstrated the presence of fecal coliforms. Total and fecal coliforms were then quantified by the Most Probable Number Technique (TIECCO, 2001). For the confirmation and presence of *E. coli*, each gassing tube was gently agitated and a loopful was streaked on Hecktoen Enteric agar and SS agar plates that were then incubated for about 24 h at 37°C. The API 20E test was used for identification.

RESULTS AND CONCLUSIONS

Results related to total and fecal coliforms quantified by the Most Probable Number Technique for each media are reported in Tables 1, 2 and 3 for mozzarella cheese, vegetable and meat, respectively.

The total coliform results were: superposition (26.92%), greater specificity of EC (46.16%), greater specificity of 2% Brilliant Green Bile broth (26.92%). With regards to the fecal coliforms, results were: superposition (57.69%), greater specificity of EC (28.85%), greater specificity of 2% Brilliant Green Bile broth (13.46%). The results confirm the greater specificity of EC broth and therefore it is suggested that EC broth should be considered as an efficient method for the determination of total and fecal coliforms in these food products.

In conclusion the specificity of culture media for the presence of coliforms

Table 1 - Total and fecal coliforms in mozzarella cheese samples using EC broth and 2% Brilliant Green Bile. Results are reported as the most probable number/g.

Samples	Total coliforms by EC	Total coliforms by 2% Brilliant green bile	Fecal coliforms by EC	Fecal coliforms by 2% Brilliant green bile
1	93	43	4	0
2	43	75	4	0
3	≥2.400	1.100	0	4
4	150	93	0	0
5	≥2.400	1.500	0	0
6	200	43	15	9
7	75	4	0	0
8	9	23	0	0
9	43	43	4	0
10	≥2.400	93	9	4
11	150	21	4	4
12	≥2.400	≥2.400	7	9
13	≥2.400	≥2.400	≥2.400	≥2.400
14	≥2.400	≥2.400	≥2.400	≥2.400
15	4	4	0	0
16	39	20	23	0
17	≥2.400	120	9	4
18	240	240	9	7
19	4	4	0	0
20	3	3	0	0

in food is very important for food microbiology since these bacteria are associated with a variety of human infectious diseases ranging from mild gastroenteric syndromes to several diseases. Therefore studies undertaken to better identify total and fecal coliforms can contribute to ensuring the sanitary quality of food.

Table 2 - Total and fecal coliforms in vegetable samples using EC broth and 2% Brilliant Green Bile. Results are reported as the most probable number/g.

Samples	Total coliforms by EC	Total coliforms by 2% Brilliant green bile	Fecal coliforms by EC	Fecal coliforms by 2% Brilliant green bile
1	≥2.400	1.500	0	0
2	240	20	0	0
3	930	15	0	0
4	3	0	0	0
5	0	23	0	0
6	150	93	0	0
7	93	23	0	0
8	43	93	15	7
9	≥2.400	≥2.400	0	0
10	210	1.100	0	4
11	75	21	0	0
12	460	150	0	0
13	≥2.400	240	23	9
14	240	210	0	0
15	≥2.400	460	4	0
16	≥2.400	≥2.400	23	23
17	≥2.400	≥2.400	210	28
18	460	1.100	0	4
19	0	43	0	0
20	4	43	0	0

Table 3 - Total and fecal coliforms in meat samples using EC broth and 2% Brilliant Green Bile. Results are reported as the most probable number/g.

Samples	Total coliforms by EC	Total coliforms by 2% Brilliant green bile	Fecal coliforms by EC	Fecal coliforms by 2% Brilliant green bile
1	≥2.400	460	23	15
2	1.100	≥2.400	240	1.100
3	1.200	≥2.400	0	0
4	≥2.400	≥2.400	9	9
5	210	240	0	0
6	1.100	1.100	0	75
7	750	430	0	0
8	150	210	7	0
9	≥2.400	≥2.400	4	7
10	21	120	0	0
11	210	460	0	0
12	1.100	1.100	0	0

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SURVEY OF HEAVY METALS IN TURKISH WHITE CHEESE

DETERMINAZIONE DEI METALLI PESANTI NEL FORMAGGIO BIANCO TURCO

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ABSTRACT

Various heavy metals were determined in commercial Turkish white cheese by atomic absorption spectrophotometry (AAS). Mean concentrations (µg/g) of Pb, Cd, Ni, Co, Cr, Cu, Zn and Fe in cheeses were 0.415, 0.127, 1.057, 0.470, 0.131, 0.629, 15.576 and 3.610, respectively. The results were compared with those reported in recent years. Overall metal levels were comparable with previous data for different cheeses, but Pb, Cd, Ni and Co levels were found to be slightly higher. The overall mean concentrations of Cr, Cu, Zn and Fe levels were in agreement with the literature data.

RIASSUNTO

Tramite spettrofotometria ad assorbimento atomico (AAS) sono determinate le concentrazioni di metalli pesanti in campioni di formaggio bianco turco disponibili in commercio. Dall'analisi è emerso che Piombo (Pb), Cadmio (Cd), Nichel (Ni), Cromo (Cr), Cobalto (Co), Rame (Cu), Zinco (Zn) e Ferro (Fe) sono presenti per un quantitativo medio, espresso in µg/g, pari rispettivamente a: 0,415, 0,127, 1,057, 0,470, 0,131, 0,629, 15,576 e 3,610. Confrontando i risultati ottenuti con quelli rinvenuti in letteratura negli ultimi anni per diversi tipi formaggio, si rileva che la concentrazione di tutti i metalli pesanti esaminati è in accordo con i dati disponibili in letteratura, eccetto che per il Pb, Cd, Ni e Co, il cui valore è risultato maggiore.

- Key words: Beyaz Peynir, cheese, heavy metals, trace element, Turkish white cheese -

INTRODUCTION

White cheeses are known by different names and similar varieties are produced in the Mediterranean countries such as Greece (Feta), Yugoslavia (Beli-Sir U-Kriskama), Bulgaria (Bjalo Salamureno Sirene), Egypt (Domiati), Israel (Brinza), Syria (Akaawi) and Turkey [Turkish white cheese, Beyaz peynir, Edirne peyniri] (ABD EL-SALAM *et al.*, 1993).

Turkish white cheese, a brined (pickled) cheese variety with a soft or semi-hard texture and a salty, acidic taste, has the highest consumption rate in Turkey. It is manufactured using a predominantly enzymatic coagulation. The curd is cut into cubes (~2 cm) which are allowed to rest in the whey for 5-10 min. They are then transferred to various sized stainless steel moulds which are lined with cheese cloth. The surface of the cheese is covered with cheese cloth, followed by a plate on which weights are placed to compact the curd; the pressure is applied at room temperature for 3-6 h or until whey drainage has stopped. The pressure is 20-40 kg weights for each 100 kg cheese milk (HAYALOGLU *et al.*, 2002). Then the cheese mass is cut with a knife into blocks weighed 350-500 g. The blocks are placed in brine (14-16 g/100 g NaCl) for 6-12 h at 15°-16°C. The brined blocks are then arranged on the bottom of a tin can (18 L), the can is filled with brine (14-16 g/100 g NaCl) and the container is closed. The cheese is ripened in the cans for 30-90 days at 6°-10°C (HAYALOGLU *et al.*, 2002). Recently, white cheeses have been produced in one or five kilogramme-tin cans full of brine which may partly explain the source of any trace contamination.

Turkish white cheese was originally manufactured from sheep or goat milk, but cow milk or a combination of milks is generally used for its production (YILDIZ *et al.*, 1989). It is produced exclusively by artisanal procedures in small dairy plants or on farms, where the quality can vary, and in modern mechanized plants in which standardized production methods are used (HAYALOGLU *et al.*, 2002).

The mineral and trace elements of the cheese depend on the mineral element content of the milk used in its manufacture and on manufacturing conditions, type of coagulation and intensity of wheying, salting and packing (MARTIN-HERNÁNDEZ *et al.*, 1992; FRESNO *et al.*, 1995).

The concentration of certain health-related elements in milk and cheese are closely dependent upon animal species and feeding, season, environmental conditions and manufacturing processes (CONI *et al.*, 1996).

Previous investigations have shown that a number of heavy metals in different dairy products might be a serious health risk for humans and animals. Therefore, it is indispensable to determine heavy metals in milk and dairy products regularly (BARBERA *et al.*, 1993; SCHUMACHER *et al.*, 1993; RAGHUNATH *et al.*, 1997; SIMSEK *et al.*, 2000).

The literature on the trace element composition of Turkish white cheese is rather scarce. The levels of Se (YANARDAG and ORAK, 1999), Pb, Cd, As and Hg, (DEMIROZU-ERDINC and SALDAMLI, 2000), Cu, Co, Ni, Mo, V, Cr, Mn, Zn, Fe and Ba (MERDIVAN *et al.*, 2004) and Ca, Mg, Na and K (DEMIRCI, 1988; ORAK *et al.*, 1998; MERDIVAN *et al.*, 2004) have been determined in some varieties of Turkish white cheese.

The aim of this study was to determine the levels of Pb, Cd, Ni, Cu, Co, Cr, Zn and Fe in Turkish white cheese from main producers and to compare them with values in the literature.

MATERIALS AND METHODS

Forty samples of Turkish white cheese produced mainly in western Turkey were analyzed for Pb, Cd, Ni, Co, Cr, Cu, Zn

and Fe. The samples ready for consumption were purchased from markets in Istanbul. The samples were stored in a refrigerator until analysis.

Cheese samples (30 g) were accurately weighed in a silica dish and dried overnight at 100°C. The samples were then dry ashed at 450°C until white ash was obtained. The ash was dissolved with 10 mL of 1 N HNO_3, transferred to a 50 mL volumetric flask, and diluted to the mark with double distilled water (JORHEM, 2000).

Nitric acid (65% by weight) (Merck) and (1 mg/mL) stock standard solutions of Pb, Cd, Ni, Co, Cr, Cu, Zn and Fe (Merck) were used. Working standard solutions were prepared by successive dilution of the stock standard solution to desired concentration in 0.2% HNO_3 as diluent.

All analyses were carried out in duplicate. Element determinations were performed with a Varian Spectra AAS 220 Fast Sequential Atomic Absorption Spectrometer (Terra Analysis and Measurement Equipment Trade Co., Inc., Ankara, Turkey) using air-acetylene flame and single-element hollow cathode lamps for each element.

In order to assess the accuracy of the procedure, a certified reference material (SRM) (LGC7160 Crab Paste – Metals), was used. The SRM was ashed, dissolved and diluted as the cheese samples. Differences between certified and experimentally found concentrations were up to 0.7-2.2% for Cu, Zn, Fe and 2.2-4.3 for Cd, Co, Ni and Cr.

RESULTS AND DISCUSSION

The results of the heavy metal content (Pb, Cd, Ni, Co, Cr, Cu, Zn and Fe) of 40 Turkish white cheese samples are presented in Table 1. The literature data for heavy metal levels in different cheeses from different countries are summarized in Table 2.

The mean values of lead and cadmium in Turkish white cheese samples were found to be 0.415±0.156 (0.216-0.759) µg/g and 0.127±0.048 (0.048-0.271) µg/g, respectively. Mean values for Pb and Cd were lower than that of Jordanian cheese (EREIFEJ and GHARAIBEH, 1993), but were higher than those of Italian (CONI *et al.*, 1995, 1996) and (ALBERTI-FIDANZA *et al.*, 2002), Finnish (VARO *et al.*, 1980), Bulgarian (KARADJOVA *et al.*, 2000) and Turkish white cheese from Ankara (DEMIROZU-ERDINC and SALDAMLI, 2000). According to the results on levels of Pb and Cd in cow milk (DEMIROZU-ERDINC and SALDAMLI, 2000; SIMSEK *et al.*, 2000), a portion of the lead and cadmium must have come from sources other than milk. The sources of high levels of Pb and Cd are likely to be the transferred from the tin can and salt used in the brine (EREIFEJ and GHARAIBEH, 1993; CONI *et al.*, 1996).

The nickel content of the cheese samples was determined to be 1.057±0.209 (0.654-1.518) µg/g and this value is lower than that found in Jordanian cheese (EREIFEJ and GHARAIBEH, 1993) and white cheese from Diyarbakir (MERDIVAN *et al.*, 2004) but higher than others in the literature (Table 2). Dairy industries use different equipment made from nickel alloys. The low quality of these alloys and the salt used in the brine may be other sources of contamination (CONI *et al.*, 1996).

Cobalt levels of cheese samples were found to be 0.470±0.164 (0.230-0.878) µg/g. This value is very high when compared to the literature (Table 2), except for white cheese from Diyarbakir (MERDIVAN *et al.*, 2004).

Chromium was determined in Turkish white cheese samples to be 0.131±0.061 (0.047-0.267) µg/g, which is less than Bulgarian (KARADJOVA *et al.*, 2000), Italian (ALBERTI-FIDANZA *et al.*, 2002), Quartirolo and Semigrasso cheeses (CONI *et al.*, 1995) and white cheese (MERDIVAN *et al.*, 2004), but is higher

Table 1 - Composition of heavy metal concentration in Turkish white cheese samples as (μg/g) fresh weight[1]. Each value is the average of 40 samples.

	Cd	Pb	Ni	Co	Cr	Cu	Zn	Fe
Mean	0.127	0.415	1.057	0.470	0.131	0.629	15.576	3.610
SD	0.048	0.156	0.209	0.164	0.061	0.253	5.249	2.167
Min	0.048	0.216	0.654	0.230	0.047	0.267	5.200	1.556
Max	0.271	0.759	1.518	0.878	0.267	1.186	27.774	8.694

[1]The mean value of dry matter of Turkish white cheese samples is 40.12%, SD: Standard Deviation, Min: Minimum, Max: Maximum.

than sheep and goat cheeses (CONI *et al.*, 1996) and Finnish cheese (VARO *et al.*, 1980). Chromium is used mainly in the production of stainless steel materials. Release from stainless steel vessels seems to be the main source of contamination (ALBERTI- FIDANZA *et al.*, 2002).

Copper content of the cheese samples was 0.629±0.253 (0.267-1.186) μg/g. The Cu content of different cheeses ranges widely. The average value for copper in Turkish white cheese was almost similar to that found in French (LAMAND *et al.*, 1994), but higher than that of American processed, and Cottage cheese (PENNINGTON *et al.*, 1986) and white cheese (MERDIVAN *et al.*, 2004), but lower than Quartirolo and Semigrasso (CONI *et al.*, 1995), Finnish (VARO *et al.*, 1980), Jordanian (EREIFEJ and GHARAIBEH, 1993) and Bulgarian cheeses (KARADJOVA *et al.*, 2000).

Turkish white cheese is produced using a predominantly enzymatic coagulation which involves a lower loss in minerals. On the other hand the intense drainage causes great losses of the minerals which are found in the soluble fraction of the whey (FRESNO *et al.*, 1995). This fact explains the moderate values of Zn in the samples. Zinc levels of cheese samples were found to be 15.576±5.249 (5.200-27.774) μg/g and this value is higher than for Cottage cheese (PENNINGTON *et al.*, 1986) and sheep and goat cheeses (CONI *et al.*, 1996), but is lower than that in other cheeses (Table 2). The acid precipitated casein contained less Zn than the curd (HARZER and KAUER, 1982). The cheese samples which contain very low Zn may be produced with acid precipitated casein instead of whole milk.

Iron levels in cheese samples were 3.610±2.167 (1.556-8.694) μg/g. This value is higher than that found in Finnish cheese (VARO *et al.*, 1980), Semigrasso, sheep and goat cheeses (CONI *et al.*, 1995, 1996), American processed and Cottage cheese (PENNINGTON *et al.*, 1986) and French (LAMAND *et al.*, 1994), but is lower than Jordanian (EREIFEJ and GHARAIBEH, 1993) and white cheese from Diyarbakir (MERDIVAN *et al.*, 2004). Turkish white cheese is normally produced from cow or a combination cow and sheep or goat milk. The cow milk cheeses showed, on average, higher amounts of Fe and Zn than goat and sheep cheeses (FRESNO *et al.*, 1995).

The levels of Pb, Ni, Cr, Cu, Zn and Fe found in this study are comparable to the levels previously found by others (DEMIROZU-ERDINC and SALDAMLI, 2000; MERDIVAN *et al.*, 2004) in Turkish white cheese.

Commercial Turkish white cheese is produced exclusively by artisanal procedures from different types of milk and with different amounts of fat in small dairy plants or on the farm, using different equipment of variable quality. The different degrees of contamination from the brine and from the equipment which are in contact with the milk and with the

Table 2 - The heavy metal concentration in cheese samples from different countries expressed as μg/g.

Country	Cheese Type	Cd	Pb	Ni	Co	Cr	Cu	Zn	Fe	Ref.
Jordan	White Cheese (Tin container)	0.4±0.09	7.8±3.4	3.1±1.2	-	-	4.6±6.5	29.6±13.0	20.8± 22.3	EREIFEJ and GHARAIBEH, 1993
Bulgaria	White Cheese	0.021	0.031	0.280	0.009	0.260	0.950	-	0.0086	KARADJOVA *et al.*, 2000
Italy	Quartirolo (Mean of 3 data)	0.044	0.127	0.276	0.045	0.718	5.694	40.9	6.67	CONI *et al.*, 1996
	Semigrasso (Mean of 4 data)	0.076	0.133	0.146	0.052	0.702	17.34	60.53	4.63	CONI *et al.*, 1996
	Sheep Cheese (Mean of 4 data)	0.117	0.105	0.015	0.018	0.055	0.995	19.5	3.27	CONI *et al.*, 1995
	Goat Cheese (Mean of 2 data)	0.114	0.077	0.007	0.0125	0.062	1.105	18.1	2.44	CONI *et al.*, 1995
	Cheese	0.018±0.008	0.075±0.031	0.347±0.179	-	0.331±0.159	-	-	-	ALBERTI-FIDANZA *et al.*, 2002
Finland	Mean values of 8 Cheese	0.007	0.062	0.05	0.009	0.032	5.25	30.9	2.10	VARO *et al.*, 1980
USA	American Processed Cheese	-	-	-	-	-	0.45	34.1	3.2	PENNINGTON *et al.*, 1986
	Cottage Cheese	-	-	-	-	-	0.16	4.1	1.1	PENNINGTON *et al.*, 1986
France	Cheese	-	-	-	-	-	0.7	29.5	2.9	LAMAND *et al.*, 1994
Turkey	White Cheese	0.022±0.17	0.260±0.51	-	-	-	-	-		DEMIROZU-ERDINC and SALDAMLI, 2000
	White Cheese	-	-	1.22±1.55	1.54±1.55	0.17±0.24	0.53±0.44	17.74±4.22	5.43±8.52	MERDIVAN *et al.*, 2004

cheese cause variability in the content of each mineral element of the different sample of cheeses. The results of this survey indicate that the concentrations of Pb, Cd, Co and Ni in Turkish white cheese were higher than that found in previous publications. Because of their toxicity, contamination with these elements needs to be further investigation in dairy products and food.

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ABSTRACTS OF DOCTORAL RESEARCH IN FOOD SCIENCE

(from the 9th workshop on Developments in
Italian Doctoral Research in Food Science and Technology,
Parma, September 8-10, 2004

Pile composting of two types of olive husk residues: quality of cured compost and biocontrol of phytopathogens
Gabriele Alfano - DISTAAM, Università degli Studi del Molise, Campobasso

In this work we propose composting as a biological treatment for olive mill residues: moist olive husks and olive leaves. One-year-old composted moist husks and sheep manure, used as inoculum to facilitate the starting phase of the process were evaluated. Trials were conducted in four piles, under different experimental conditions (turnover, static, type of inoculum). The best results were obtained with aeration and enough inoculum to induce a fast start-up and correct evolution of the composting process; the final cured compost was hygienically clean. Water extracts from composted residues (at the 90th day) exhibited a clear inhibitory-suppressive activity toward *V. dahliae* and 8 phytopathogenic fungi.

Thermo-mechanical properties of dough influence of sodium chloride, mixing time and equipment
Alessandro Angioloni - Dipartimento di Scienze degli Alimenti, Università di Bologna, Cesena (FC)

Thermo-mechanical properties of common wheat dough obtained with different kneading conditions and with different amounts of sodium chloride were investigated. Dough mixing is important in the preparation of any kind of bakery product and the added ingredients such as salt, also have very important functions in the process. Mechanical thermal analysis showed that the high-speed mixing conditions and the addition of salt to the dough slowed heat-induced reactions such as starch gelatinisation and protein coagulation.

Microbiological and molecular characterization of sourdough in Sardinia
Francesca Balzano - Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, Università degli Studi di Sassari

Sourdough, or natural yeast, used in bread-making processes, is a complex ecosystem which contains both yeasts and lactic bacteria. Sourdoughs used in the production of typical bread from all areas of Sardinia were analyzed. Isolated microorganisms were characterized and selected in order to produce a starter that is the most similar to the natural one and that will assure the senso-

ry features of traditional bread. Moreover RAPD-PCR was used in the molecular characterization of the yeast strain in order to detect probable biodiversity.

Antimicrobial effect of essences used in soft drink production

Nicoletta Belletti - Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna

The aim of this study was to identify and characterize the microorganism responsible for a case of industrial spoilage in a soft drink factory. About 500,000 bottles of an orange-based soft drink were fermented by a *Saccharomyces cerevisiae* strain in spite of the addition of sodium benzoate. A similar soft drink, which had a different flavoring component, did not spoil because of the antimicrobial activity of the orange essence used in its production.

Tyrosine decarboxylase activity of *Lactobacillus pentosus*

Antonio Bevilacqua - Dipartimento di Scienze degli Alimenti, Università di Foggia

The influence of pH, glucose and NaCl concentration on tyrosine decarboxylase activity of a strain of *Lactobacillus pentosus* was studied. Results pointed out that pH, glucose and NaCl concentration significantly influenced tyrosine metabolism.

Integrated cell culture-PCR (ICC/PCR) in virological research on environmental samples and on food

Laura Bigliardi - Dipartimento di Salute Pubblica, Università di Parma

A study was started to evaluate the efficacy of ICC/PCR for isolating and identifying cell culture cultivable viruses in low concentrations in environmental samples and in food. Poliovirus 1 and HAV were assayed. The lowest concentration of the two viruses was calculated by PCR; the first viral PCR negative concentration was inoculated in cell culture to follow the viral replication over time. The supernatant and the cell culture lysate were assayed by PCR to analyse the time needed to give positive results. For Polio 1 the first results showed times of 72 hours for cell culture lysate and for HAV the times were 72 hours for lysate and 96 hours for supernatant.

Liquid chromatography-electrospray-QQQ-MS/MS and capillary liquid chromatography-electrospray-QTOF MS for the determination of Sudan azodyes in hot chilli products

Francesca Calbiani - Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma

The Commission decision 2003/460/EC and its implementation of 21 January 2004 on emergency measures concerning hot chilli and hot chilli products intended for human consumption require the development of reliable and accurate analytical methods for the identification and quantification of Sudan I, II, III and IV in foodstuffs. In this work, an accurate method for determining these analytes in hot chilli food samples based on the use of reversed-phase liquid chromatography-electrospray-tandem mass spectrometry was devised and validated in-house. The potential of capillary liquid chromatography-quadrupole/time-of-flight mass spectrometry to confirm the identity of these contaminants in hot chilli products was demonstrated.

Qualitative assessment of lactose-hydrolysed milk

Tiziana Candigliota - Dipartimento di Scienze e Tecnologie Agro-alimentari Ambientali e Microbiologiche, Università degli Studi del Molise, Campobasso

A lactose-hydrolysed milk during processing was assessed by carbohydrate analysis (glucose, lactose and galactose) and by some process markers (furosine, lactulose and fructose) in order to monitor the intensity and the sequence of thermal and/or hydrolysis treatments. The results obtained show that the use of these markers (single or in combination) allows the technology to be characterized and the quality of lactose-hydrolysed milk to be assessed.

Microbial shelf-life determination of chilled buffalo meat treated with lysozyme and EDTA

Marianna Cannarsi - Dipartimento di Scienze degli Alimenti, Università di Foggia: Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la Valorizzazione dei Prodotti Tipici e di Qualità, Università di Foggia

The effectiveness of lysozyme alone or in combination with EDTA (disodium ethylenediaminetetraacetate salt, a chelating agent active against the Gram- bacteria), in inhibiting the growth of spoilage microorganisms in buffalo meat is addressed. Samples were dipped either in solutions containing different concentrations of lysozyme (0.5, 1 and 2%) or in solutions containing a pre-fixed quantity of lysozyme (0.5%) and increasing concentrations (0.5, 1 and 2%) of EDTA. The best results, in terms of shelf life extension, were obtained by dipping the meat in a solution containing a combination of 0.5% lysozyme and 2% EDTA. This treatment reduced the growth of all the investigated bacteria and showed a bactericidal effect on *B. thermosphacta* during the entire storage time.

Detection of biogenic amines in food products by an innovative amperometric biosensor

Donatella Carelli - Dipartimento di Scienze degli Alimenti, Università degli Studi di Foggia

Biogenic amines are compounds used as quality marker analytes in food because of their influence on human health if present at high concentrations. This work reports the construction of an amperometric biosensor for determining biogenic amines, by using commercial diamino-oxidase as biocomponent, entrapped by glutaraldehyde onto electropolymerised bilayer polymers. In spite of very low enzyme activity, the biosensor displayed a high response sensitivity in flow experiments, a good linear response and low detection limits. The excellent anti-interference characteristics allow the biosensor to be used in the analysis of food products.

Total composition of sterols in extra virgin olive oils obtained with different extraction technologies and their influence on the oxidative stability of the oil

Luisito Cercaci - Dipartimento di Scienze degli Alimenti, Università di Bologna

The composition and antioxidant capacity of total sterols of extra virgin olive oils obtained with different extraction technologies from olives harvested at two ripening stages were studied. The antioxidant capacity was evaluated with the Oxidative Stability Instrument (OSI), by using a model system made of commercial refined peanut oil (which was previously treated in order to reduce the polar compounds) and enriched with the total sterol fractions.

Effect of adding citric acid, during malaxation, on the oxidative stability of produced, unrefined olive oil

Lorenzo Cerretani - Dipartimento di Scienze degli Alimenti, Università di Bologna, Cesena (FC)

Extra virgin olive oil has a higher oxidative stability than other edible oils. This peculiarity is due to its fatty acid composition and the high content of antioxidant compounds. In particular, phenols are the main natural antioxidants of virgin olive oil, although there are other, less polar, substances such as tocopherols and carotenoids that show an antioxidant activity. This work evaluated the possibility of increasing the phenolic compounds content, by adding a technological adjuvant (citric acid) during the malaxation phase of olive oil production, with the aim of increasing the oxidative stability and nutritional value of the oil.

Prediction of perceived astringency induced by phenolic compounds by means of an in vitro assay

Nicola Condelli - Dipartimento di Biologia, Difesa e Biotecnologie Agroforestali, Università degli Studi della Basilicata, Potenza

In this work a new method for estimating perceived astringency is presented. Twenty-four subjects were selected on the basis of having similar salivary flows. Subjects were asked to rate the perceived astringency of phenolic solutions. In vitro assays were performed with the same astringent solutions and the reactivity phenol-mucin expressed in terms of NTU. Predictive curves described by linear regression of astringency intensity vs NTU were found. The predictive capacity of the models was tested by comparing measured and predicted astringency intensity in a set of samples. The predictive model was also applied to evaluate the astringency of 18 red wines. A linear relationship was found between the astringency of wine samples and the *in vitro* assay response.

Barrier properties of edible films

Amalia Conte - Dipartimento di Scienze degli Alimenti, Università degli Studi di Foggia

A study on the influence of the solubilization and diffusion process on the barrier properties of four different edible films is presented. A mathematical model was fitted to the experimental data to obtain quantitative information on both the solubilization and diffusion process. Results suggest that the different water and oxygen permeability coefficients of the films have to be ascribed to their different affinity to water and macromolecular mobility.

Determination of conjugated linoleic acid (CLA) content in typical Apulian cheeses: effects of cheese processing and aging

Monica Dentico - Dipartimento di Scienze degli Alimenti, Università degli Studi di Foggia

It has been demonstrated that conjugated linoleic acids have numerous physiological functions; in particular, some of them seem to have potential health-promoting properties. Due to the importance of these actions, the aim of this work is to determine CLA by silver-ion high-performance liquid chromatography (Ag+-HPLC) in some typical Apulian cheeses: mozzarella (obtained with starters and citric acid), caciocavallo (mild and spicy), caciotta and murgetta. All samples showed a high content of 9cis, 11trans-octadecadienoic acid (rumenic acid), an active isomer that has been associated with important biological activities. Furthermore, CLA content increas-

es during the manufacturing processes and is strictly dependent on the production protocol.

Glycemic variation after ingestion of sourdough bread

Mariella Dettori - Dipartimento di Scienze Ambientali Agrarie e di Biotecnologie Agro-Alimentari, Università di Sassari

Fermentative processes of sourdoughs are produced by the complex action of yeasts and lactic acid bacteria. The action of these microorganisms, beside giving different sensorial characteristics to the bread, changes the digestibility and the glucidic availability of the bread. This study presents the preliminary data concerning the modifications of the post-prandial glucose response in normal patients after ingesting bread obtained with different fermentative processes and different leavening times.

Determination of minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of 12 plant essential oils against food-borne pathogen and non-pathogen bacteria

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Essential oils extracted from 12 different aromatic plants were tested for their antimicrobial activity against pathogen and non-pathogen bacterial strains, both Gram-positive and Gram-negative. For each essential oil the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) were determined.

The results showed that the Gram-negative strains were more sensitive than Gram-positive ones, in particular *Escherichia coli* and *Salmonella* were sensitive to all oils while, among the Gram-positive, *Brochotrix thermosphacta* was the most sensitive. The lactic acid bacteria showed a high resistance, in fact, only a few oils caused their inactivation but always at concentrations above 1%.

Winemaking processes to emphasize the link between grape cultivar and production zone

Silvia Falbo - Istituto di Enologia e Ingegneria Alimentare, Università Cattolica del Sacro Cuore, Piacenza

The winemaking processes now in use, derived from both tradition and the need to process large quantities of grapes, are not always suitable to highlight the link between vine variety and production zone. Towards this goal, winemaking tests regarding three typical Colli Piacentini vine varieties such as Malvasia di Candia Aromatica, Barbera and Croatina, were carried out using various techniques.

Development and quality assessment of functional pastas

Rita Fanelli - Dipartimento di Scienze e Tecnologie Agro-alimentari Ambientali e Microbiologiche, Università degli Studi del Molise, Campobasso

Two different types of functional pastas were obtained by adding i) barley flour enriched wich β-glucans, and ii) inulin to the semolina. The viscoelastic properties of dough and pasta cooking quality were assessed. Both functional pastas showed excellent cooking quality due to the balanced formulations and the appropriate technology used.

Extraction of antioxidants from natural sources and food wastes

Francesco Fusca - Istituto di Enologia e Ingegneria Alimentare, Università Cattolica Sacro Cuore, Piacenza

The organic wastes of the oenological industry (grape stalks, pomace, lees and water), even if not dangerous, can cause serious environmental problems if they are not properly disposed. Many substances could be extracted from these wastes to be used in different fields, in particular polyphenolic compounds which exhibit antioxidant and anticarcinogenic properties. The aim of this work was the recovery of polyphenolics from grapestalks and pomace. The efficiency of two solvents (ethanol and ethyl acetate) was evaluated at different times and temperatures of maceration, and in the presence of ultrasound and vitamin C.

Genetic and cytochemical differentiation of strains of *Staphylococcus xylosus*

Vincenzina Fusco - Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli “Federico II”, Portici (NA)

Staphylococcus (S.) xylosus plays an important role in fermented meat products, mainly for its nitrate reductase, catalase and superoxide dismutase activities. With the aim of providing reliable analytical procedures for detecting and monitoring such microorganism, at the species and strain level, 31 *S. xylosus* strains from different environments were characterised using a polyphasic approach.

Influence of emotions and memory on consumer food choices

Nicola Gabbiadini - Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano

Different studies were conducted to investigate the relationship between food and emotions and to understand if and how consumers' behaviour and food choices are modified in different emotional conditions. Eating behaviour was investigated under different emotive conditions using 2 questionnaires and 90 subjects. The influence of four basic emotions (joy, sadness, anger and fear) of consumers' personality and memory on food choice, and place and modalities of consumption was studied. The results show different food behaviour according to the emotion considered; there were also some gender differences.

Effects of microwave heating on oil in vegetable creams

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Extra virgin olive oil and asparagus cream containing 33% extra virgin olive oil were exposed to microwave radiation for several periods of time and different power intensities in order to study oil oxidation. A microwave oven with a frequency of 2450 MHz was used. The evaluation of thermal damage was based on peroxide values and the Rancimat test as an indication of primary or secondary oxidation. The first steps of microwave heating did not promote lipid oxidation.

Differentiation between *Bacillus cereus* and *Bacillus thuringiensis* using a PCR-RE analysis

Cristina Giusto - Dipartimento di Scienze degli Alimenti, Università di Udine

Bacillus species are of industrial and clinical relevance, since the production of different kinds of toxins by *B. cereus* and *B. thuringiensis* which has been associated with illness due to the ability of the toxins to induce necroses of human tissues or gastro-intestinal infections. The possibility of differentiating between *B. cereus* and *B. thuringiensis* to prevent intoxication, monitoring potentially contaminated foods is a

real need. Comparison of *B. cereus* and *B. thuringiensis* DNA is a tool to obtain clearer results than classical microbiological methods. A Polymerase Chain Reaction (PCR) followed by Endonuclease Restriction (RE) were able to achieve the differentiation needed in spite of the high similarity shown by these closely related species, *B. cereus* and *B. thuringiensis*.

Effect of blending durum wheat cultivars of different strength on dough viscoelastic properties

Luisa Gracco - Dipartimento di Scienze degli Alimenti, Università di Udine

The influence of blending durum wheat cultivars of different strength on dough viscoelastic properties was evaluated using large strain (alveograph and farinograph) and linear dynamic rheological measurements. The work was supported by size-exclusion high-performance liquid chromatography (SE-HPLC) analysis of the relative amounts of glutenin and gliadin protein fractions.

Quali-quantitative evaluation of water buffalo milk fat: influence of feeding condition and seasonal nature and setting up of a method for evaluation of water buffalo milk fat purity

Gianfranco Lambiase - Dipartimento di Scienze degli Alimenti, Università di Napoli, Portici (NA)

Food authenticity has become a focal point attracting the attention of producers, consumers and policy makers. One of the targets of fraudulent action is oils and fats. Butter, concentrated butter, and milk fat in general can be illegally adulterated with less expensive vegetable fats or animal fats. Bovine milk fat purity is evaluated by the gas chromatographic determination of triglycerides (TG) performed by using the official method of the European Community (Precht method). This method permits the detection of foreign fats, of both animal and vegetable origin. The aim of this work was to propose a method for evaluation of water buffalo milk fat purity after standardization of the buffalo milk fat. The results show that using the modified Precht method, it is possible to determine the foreign non lactic fat at 2-5% in water buffalo milk fat.

Scale-up of a single-screw extruder for pasta: effect of die shape on operating parameters

Augusto Langella - Dipartimento di Scienze degli Alimenti, Università di Napoli, Portici (NA)

Scale-up is the task of producing an identical process result at a larger scale than previously accomplished. This work presents the derivation of a set of scale-up rules suitable for food extrusion technology, particularly for pasta production. The principal factors affecting a pasta extrusion process are the material properties, the barrel and die temperature, the extrusion ratio and the die shape. The extruder die plays an important role in the extrusion process. The aim of this work was to study the effect of die design on operating parameters of pasta extrusion in order to define primary and secondary scale-up criteria.

Improvement of rheological and fermentative performance by addition of enzymes to flour

Alberto Molinari - Dipartimento di Protezione e Valorizzazione Alimentare, Università di Bologna

The study was carried out to investigate the effects of commercial enzymes (amylases) and process variables such as NaCl and pH on dough performance in breadmaking processes. The main properties investigated were Falling Number measured by the Hagberg instrument, dough development and gaseous re-

lease measured with a Rheofermentometer and production of fermentable sugars (maltose, glucose and fructose) determined by HPLC. Enzymes at different concentrations (5-50 g/ql) were tested in order to optimise yeast cell increase, leavening performance and ethanol production.

Food chain traceability of lactic acid bacteria in dairy products

Marco Ambrogio Murgia - Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, Università degli Studi di Sassari

The norms of food chain traceability impose several actions aimed at reconstructing the history of a food, as well as each of its components, along the food chain line. For this aim autochthonous lactic acid bacterial strains were isolated from typical Sardinian cheeses and subsequently classified and selected with regard to their technological features. The goal was to make up and use a starter culture in experimental trials and, subsequently, to verify the real traceability of the strains using molecular tools such as RAPD-PCR.

Antagonism between a flor strain of *Saccharomyces cerevisiae* and *Penicillium expansum* on stored apple fruit

Giuseppe Ortu - Dipartimento di Protezione delle Piante, Centro interdisciplinare per lo sviluppo della ricerca biotecnologia e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agroalimentari, Università di Sassari

The film-forming *S. cerevisiae* strain M25 showed significant ability to reduce postharvest decay on apple caused by *Penicillium expansum*. The biocontrol effect depended on the medium, the growth conditions, and the stage at which the yeast was collected and inoculated into artificial wounds.

Yeasts in the sourdough bread of Marche region

Andrea Osimani - Dipartimento di Scienze degli Alimenti; Università Politecnica delle Marche, Ancona

Traditional bread-making based on the use of "sourdough" as the leavening agent is largely used for the production of different types of bread and other bakery products. The aim of this study was to identify 113 yeasts isolated from sourdoughs and flours from the Marche region. Most isolates were identified using RFLP (Restriction Fragment Length Polymorphism). Automated DNA sequencing of the amplicons was carried out to discriminate isolates with unidentifiable RFLP profiles. The results obtained showed the presence of the three species *Saccharomyces cerevisiae* (90%), *Candida humilis* (3%) and *Candida lusitaniae* (7%).

Microbiological characteristics of Carmasciano cheese: a traditional pecorino cheese from Irpinia

Susanna Pacifico - DISTAAM, Università degli Studi del Molise, Campobasso

Many raw milk home-made cheeses are characterized by a complex bacterial microflora that confer important features of uniqueness to the end products. The composition of the microflora, that is available inside and on the surface of these kinds of cheese, results from both autochthonous microflora and cheese-making. The aim of this work was to study the autochthonous microflora of Carmasciano, a typical pecorino cheese (or sheep's milk cheese) produced in Irpinia (Campania, Italy). The trend of the microbial population was

studied during the manufacturing and ripening of the cheese, with particular reference to the isolation of lactic acid bacteria and the identification of lactobacilli.

Protein composition, prolamin aggregation and mixing properties of genetically modified (GM) durum wheat lines, showing an altered trafficing and deposition of seed storage proteins in protein bodies

Lucia Padalino - Dipartimento di Scienze degli Alimenti, Università degli Studi di Foggia, Facoltà di Agraria, Foggia; Istituto Sperimentale per la Cerealicoltura, Sezione Operativa di Foggia

In this work significant changes were shown in the protein composition, amount of soluble and insoluble polymers formed, gluten index and mixing properties of a genetically modified durum wheat line in which the transport and deposition of protein vesicles from the Endoplasmic Reticulum to the Golgi was down-regulated by introduction of a trans-dominant mutant form of tobacco rab1 cDNA driven by an endosperm specific HMW glutenin subunit promoter.

Effect of polyphenols on lipid oxidation of horse mackerel *(Trachurus trachurus)* during thermal treatment

Cristina Parisini - Dipartimento di Scienza degli Alimenti, Università "Federico II" di Napoli, Portici (NA)

Lipid oxidation is a major cause of food deterioration with formation of several degradation compounds and relative quality loss. The aim of this work was to study the effect of polyphenols from Italian monovarietal extra virgin olive oil (cv. Coratina) on the thermal degradation of horse mackerel muscle (*Trachurus trachurus*). For this purpose the compounds produced in the oxidation phases, such as peroxide value and decomposition products of lipid peroxides were monitored.

Analysis of aroma compounds in ewe cheese from the Reggio Emilia mountain

Sara Pignedoli - Dipartimento di Protezione e di Valorizzazione Agroalimentare, Università di Bologna, Reggio Emilia

This work concerns ewe cheese production in a locality on the Apennines surrounding Reggio Emilia. During part of the year of 2003, cheesemaking was carried out, changing a few production parameters, in order to obtain a range of distinct cheeses which differed from one another because of the different milk thermic treatments and the kind of starters used. The different cheeses obtained were then analysed for their macroscopic composition, but, above all, their aromatic characterization was carried out by gas chromatography. This kind of analysis made it possible to observe the difference between the aroma compounds which characterize the ewe cheese, where the main fraction is due to the free fatty acids which were quantified by means of a pie chart.

Measuring food school lunch preferences among 4 to 6-year-old children

Sonia Policastro - Dipartimento di Biologia, D.B.A.F., Università della Basilicata, Potenza

Preschool-age children's preference for school lunch food and the relationship between their observed consumption of, and preferences for school lunch food was studied. Children rated acceptability scores for 8 first courses, 14 second courses and 9 vegetables on a 7 point facial hedonic scale, 2 hours before lunch time. The observed amounts were re-

corded by the weighing technique at the end of the meal. Results show that children's preference scores for the first and second courses had an average of 5 (just a little good), whereas the vegetables, except for potato puree, were rejected. Also, results indicated a significant relationship between consumption (p=0.000): as preferences increased, amounts not eaten decreased.

Phenolic profile of extra virgin olive oils extracted by whole and stoned Coratina olives

Maria Assunta Previtali - Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, Foggia (FG)

The phenolic content and composition of Coratina extra virgin olive oils was determined. The oils were extracted by a continuous system at three phases from whole and stoned olives which were harvested at two different degrees of maturation. The results show a higher phenolic content in the stoned oils, whereas all oils showed a very similar profile. The oils from stoned olives submitted to 45 minutes of malaxation gave more balanced sensorial characteristics. Principal components analysis (PCA) showed a clear differentiation among the oils extracted from olives at two different degrees of ripening.

Hardware-software system for the determination of food thermal diffusivity in unsteady-state heating transfer

Massimiliano Rinaldi - Dipartimento di Ingegneria Industriale, Area di Tecnologie Alimentari, Università degli Studi di Parma

A hardware-software system for measuring the thermal diffusivity in foods was developed. The thermal diffusivity was determined with software developed with Matlab® language that solved Fourier's equation using the explicit finite differences method. Using the heat penetration curves obtained experimentally it was possible to calculate thermal diffusivity values with good approximation. The proposed method was compared to the traditional way of calculation using heat penetration curves. Data obtained simulating heat exchange were very similar to the experimentally determined ones.

Critical parameters of this way of measurement were also investigated and defined.

Valorization and characterization of officinal plants in Southern Italy

Carmela Benedetta Romeo - Dipartimento di Biotecnologie per il Monitoraggio Agro-Alimentare ed Ambientale (BIOMAA), Università degli Studi Mediterranea di Reggio Calabria

The volatile compound fraction of the essential oil belonging to *Origanum* spp. of Southern Italy was examined. This medicinal and aromatic plant is known for many beneficial effects for human health. Such effects can be correlated with the activity of the chemical compounds contained in the essential oil, so the identification and knowledge of these components is very useful and interesting; moreover, the use of advanced molecular techniques can help match and trace the path from the plant to the essential oil chemotype, and new packaging and storage procedures can help minimize loss of active components after harvesting.

Interactions between meat spoilage microbial populations: behaviour of *Brochotrix thermosphacta*

Federica Russo - Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Portici (NA)

Spoilage microbial flora of fresh meat stored aerobically at 5°C for 7, 10 and 18 days were enumerated, isolated and concentrated. The predominant bacteria associated with spoilage of refrigerated beef are *Brochotrix thermosphacta*, *Enterobacteriaceae*, Lactic Acid Bacteria (LAB) and *Pseudomonas* spp. The interactions between *B. thermosphacta* and the other spoilage microbial flora were studied on meat-like medium (MM) at 5°C. The results showed that *B. thermosphacta* is generally the dominant organism when inoculated with a mix of *Pseudomonas* spp., LAB and *Enterobacteriaceae*. Moreover, there was a decrease in the growth of *B. thermosphacta* in the presence of LAB at 5°C.

Relationship among dietary habits, insulin resistance and oxidative status in a group of healthy subjects

Sara Salvatore - Dipartimento di Sanità Pubblica, Università degli studi di Parma

Several studies carried out in the last years have demonstrated that antioxidant rich and low Glycemic Index (GI) diets are a possible way to reduce the risk of insulin resistance(IR), a principal initiating factor in the development of type II diabetes. Two food frequency questionnaires were used (FFQs) to estimate diet GI, Glycemic Load (GL) and Total Antioxidant Capacity (TAC) in a cross-sectional study to identify possible correlations among dietary components, redox status and IR of subjects. Sex and BMI are determining factors in developing IR and dietary factors, such as carbohydrate quality and quantity may be associated with insulin sensitivity. Plasma lutein seemed to be inversely correlated to IR. The two FFQs proved to be useful tools to investigate the correlation among GI, TAC and IR in non-diabetic subjects.

Influence of diet and lamb slaughtering age on the composition of rennet paste used for "Pecorino Foggiano" cheese-making

Antonella Santillo - Dipartimento DISA, Università di Foggia

Experimental rennet pastes were obtained from lambs subjected to three different rearing systems (ewe rearing, artificial rearing, and artificial rearing with *Lactobacillus acidophilus* supplementation ARBL) and two different slaughtering times (20 d and 40 d). Lamb rennet pastes were prepared according to local shepards' tradition. All ripened rennet pastes showed a satisfactory microbiological quality due to the absence of coliforms and total mesophilic count <5 log10 cfu/g. Rennet pastes from ARBL displayed higher ($P<0.001$) lactobacilli counts on MRS agar (5.7log10 at 20 d and 4.6log 10 cfu/g at 40 d) than the other groups. No effects of slaughtering age were found on experimental coagulation time and dairy coagulation time for ARBL rennet pastes.

Microchip capillary electrophoresis for food characterization

Matteo Scampicchio - Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano

A microchip capillary-electrophoresis with electrochemical detection is performed for rapid and effective measurement of food compounds. Several standard mixtures of phenolic acids, amino acids, seleno-amino acids were detected and successfully resolved. Parameters of the chip separation and amperometric detection were examined and optimized. Under optimum conditions responses were achieved within 300 s. The new microchip protocol offers great promise for a wide range of food applica-

tions requiring fast measurements and negligible sample use. Besides, a former and promising application to classification of foods was performed and validated, combining high speed separation and chemometric tools toward the microchip.

Lactic acid bacteria employed in malolactic fermentation

Giuseppina Scollo - Dipartimento di Orto-Floro-Arboricoltura e Tecnologie Alimentari, Facoltà di Agraria, Università degli Studi di Catania

Lactic Acid Bacteria (LAB) isolated from grapes, musts and wines from different cultivars and wineries were studied in order to individuate strains to use as starter for malolactic fermentation. Strains from different years were identified at the species level by phenotypic methods. Some of them (86) were identified by molecular methods based on Restriction Fragment Length Polymorphism (RFLP) of 16S ribosomal DNA. A group of 17 strains was further tested for the ability to grow at different environmental conditions (pH, temperature and ethanol concentration) and for the capacity to carry out malolactic fermentation.

Phlorin as a quality marker for commercial orange juices and beverages

Monica Scordino - Dipartimento di Orto-Floro-Arboricoltura e Tecnologie Agroalimentari, sez. Tecnologie Agroalimentari, Università di Catania

An analytical procedure was developed for determining the content of phlorin (3,5-dihydroxyphenyl β-D-glucopyranoside), a marker of orange juice adulteration, in terms of corresponding aglycon phloroglucinol after a total enzymatic hydrolysis of the sample. Its quantification was performed in several commercial blond and pigmented orange juices and 12% orange-based beverages. The content of phlorin in the pigmented juices was higher than in the blond ones. In orange-based beverages the phlorin content showed a large heterogeneity of results and the poor quality of the raw juices used.

Use of advanced molecular biological techniques for typical food products from Southern Italy

Rossana Sidari - Dipartimento di Biotecnologie per il Monitoraggio Agro-Alimentare ed Ambientale (BIOMAA), Università degli Studi Mediterranea di Reggio Calabria

The aim of this study was to characterize technologically and genetically food products from local areas in Southern Italy. This project included several raw materials; our first effort was to investigate local *Olea europaea* cultivars and their extra virgin olive oil in order to ascertain relationships between cultivars and trace a genetic profile for monovarietal virgin olive oils. This profile could lead the way for a standardized certification of virgin olive oils. Studies are also going on for the implementation of similar protocols on other natural products.

Application of FT-NIR spectroscopy to study the shelf life of dairy products

Nicoletta Sinelli - Dipartimento di Scienze Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano

Food "freshness", and in particular dairy "freshness" is more and more demandad by the consumer. The shelf life of the product is negatively influence by the storage temperature. The aim of this work was to study the shelf life of two dairy products (Crescenza cheese and butter), using FT-NIR spectroscopy. The ability of FT-NIR spectroscopy was ver-

ified in order to evaluate the quality of these products during storage in relationship to the evolution of chemical and physico-chemical parameters. The main advantage of FT-NIR spectroscopy is to rapidly draw a profile of products relating to their total quality.

Typing of *Saccharomyces* interspecific hybrids by trascriptome analysis and mitochondrial proteins profile study

Lisa Solieri - Dipartimento di Scienze Agrarie, Università degli Studi di Modena e Reggio Emilia, Reggio Emilia

In the present work, the trascriptomes of parental species, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*, and of two interspecific hybrids with the mtDNA type, respectively, of *S. cerevisiae* and *S. uvarum* were investigated. When the hybrid with *S. uvarum* mtDNA was compared to the *S. cerevisiae* strain, hybrid genes involved in glycolysis pathways were upregulated. The hybrid with *S. cerevisiae* mtDNA showed downregulated genes of aerobic respiration, when compared to the *S. uvarum* strain. Finally the glycolitic pathway is inhibited in the hybrids with *S. cerevisiae* mtDNA more than in that with *S. uvarum* mtDNA. Regarding mitochondrial proteins, the mitochondrial protein profile, obtained by isoelectric focusing (IEF) and 2-D polyacrylamide gel electrophoresis (PAGE), resulted from the recombination between the genomes of the two parental strains.

Chemical-physical characterization of crystalline grape sugar

Graziana Terrasi - Dipartimento di Scienze degli Alimenti, Alma Mater Studiorum Università di Bologna

The aim of this research was the chemical-physical characterization of high concentrated (80°Brix) Rectified Concentrated grape Must (RCM) as raw material for crystalline grape sugar production.

Polyphenol oxidase determination in eggplant

Aldo Todaro - Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari (DOFATA), Università degli Studi di Catania

Enzymatic browning is one of the most important colour reactions that affects fruit and vegetables. It is catalysed by the enzyme polyphenol oxidase PPO (EC1.10.3.1) which is also referred to as phenoloxidase, phenolase, monophenol oxidase, diphenol oxidase and tyrosinase.

In this paper a specific method for PPO extraction from eggplant pulp was studied, optimizing a preceding enzymatic assay.

Cloning and sequencing of the sakP operon from *Lactobacillus sakei* isolated from natural fermented sausages

Rosalinda Urso - Dipartimento Scienze degli Alimenti, Università degli Studi di Udine

In this study 600 LAB strains isolated from naturally fermented Italian sausages, coming from three different factories of Friuli Venezia Giulia were tested for their potential ability to produce bacteriocins. *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 were selected as indicator microorganisms in the agar-diffusion method. Only six isolates showed bacteriocin activity against *L. monocytogenes* after treatment with proteinase K and neutralization. They were all identified as *Lactobacillus sakei* by molecular methods and the gene encoding the bacteriocin was identified. On

the basis of the information already available on other *L. sakei* bacteriocin-producing strains, the sakP operon was cloned and subsequently sequenced.

Characterisation of the yeast population involved in the production of a sweet leavened baked good

Pamela Vernocchi - Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna

Eighty-six strains were isolated from samples collected during the production of Colomba, a traditional Italian sweet leavened baked good. They were characterized by phenotypic methods and identified by RAPD-PCR as *Candida milleri* and *Saccharomyces cerevisiae*. Cluster analysis of RAPD-PCR profiles indicated considerable polymorphism among the isolates. Analysis of fermentation products and sugars revealed high amounts of acetic acid and mannitol after sourdough fermentation. *C. milleri* was the dominant species in the sourdough.

Lipid biosynthesis in *Saccharomyces cerevisiae* wine strains in anaerobiosis

Giacomo Zara - Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, Università di Sassari

Anaerobiosis as a cause of stuck fermentation has been scarcely investigated. However oxygen is essential for lipid biosynthesis and, thus, for cell growth and resistance to stress conditions. The lipid content and ACC1, ACS1 and ACS2 gene expression were analyzed in *S. cerevisiae* wine strains with different susceptibility to stuck fermentation.

Identification and characterization of lactic acid bacteria isolated from "Cornetto di Matera" sourdough bread

Teresa Zotta - Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Potenza

Forty-five strains of lactic acid bacteria (LAB) isolated from wheat sourdoughs, used to produce Cornetto di Matera bread, were identified by the SDS-PAGE technique and screened for their technological properties including proteolytic activity, exopolysaccharide (EPS) production and antimicrobial activity. Adaptive stress response to acid, NaCl and H_2O_2 concentrations normally lethal to LAB was investigated. Several species of LAB were found. Isolates of *Lactobacillus (Lb) plantarum* and *Lb. pentosus* exhibited good technological properties, while all strains of *Lb. curvatus* were stress intolerant and unable to produce EPS. Isolates of *Weissella (W) confusa* and *Leuconostoc (Leuc) mesenteroides* were successful in producing EPS, but did not show any antimicrobial activity.

Chemistry and Technology of Soft Drinks and Fruit Juices Second Edition

Edited by Dr Philip Ashurst, Consulting Chemist to the Food Industry, Hereford, UK
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A completely revised and expanded edition of a highly successful volume. New chapters are included on fruit and juice processing, carbohydrates and intense sweeteners, trends in beverage markets, functional beverages and non-carbonated products, and the coverage has been further extended to include carbonated products.

The second edition will be welcomed as a source of up-to-date, concentrated information on these important sectors of the food industry.

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5. Other beverage ingredients.
6. Non-carbonated beverages.
7. Carbonated beverages.
8. Processing and packaging.
9. Packaging materials.
10. Analysis of soft drinks and fruit juices.
11. Microbiology of soft drinks and fruit juices.
12. Functional beverages.
References.
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Postharvest Biology

By Stanley J. Kays and Robert E. Paull
568 pages, 285 illustrations, hardbound, 2004
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Postharvest Biology is a completely revised, expanded, and up-dated edition of the postharvest classic – Postharvest Physiology of Perishable Plant Products. The text focuses on the environmental factors modulating and functional processes controlling the physical and chemical changes in live plants and plant products after harvest. It provides a concise overview of the theoretical principles and processes governing these changes. Examples include an extremely diverse cross-section of perishable plant products (fruits, vegetables, agronomic crops, etc.).

Postharvest Biology is the one-stop reference text for Food Scientists interested in the biology of living plant products after harvest. Included is extensive information on the basic principles governing perishable crops from the gene regulation of fruit development and ripening to thermal properties (heat trans-

fer and removal), movement of gases, solutes and solvents within harvested products to desiccation and drying. Hundreds of references and illustrations are provided in keeping with the objective of providing a comprehensive overview of the topic coupled to sources for additional reading that facilitates the exploration of specific aspects in even greater detail.

A new chapter (Molecular Genetics, Signal Transduction and Recombinant DNA) has been added, reflecting the extensive changes that have occurred in our understanding of plant biology over the past decade. The chapter includes critiques on the public's concern and the advantages and disadvantages of GM crops. Also added are a number of appendices with extensive reference information (e.g., recommended storage conditions, respiratory rates, ethylene sensitivity, etc.).

Contents:

- Science and Practice of Postharvest Plant Biology;
- Nature and Structure of Harvested Products;
- Metabolic Processes in Harvested Products;
- Secondary Metabolic Processes and Products;
- Molecular Genetics, Signal Transduction and Recombinant DNA;
- Development of Plants and Plant Organs;
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Technology of bottled water
Second edition

Edited by Dorothy Senior, Group Technical Adviser, Highland Spring Ltd, Perthshire, UK;

Nicholas Dege, Supply Chain Manager, NestléWaters North America, Calistoga, California, USA

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The bottled waters industry has become a vital and vigorous sector of the beverage world, in developed and developing countries worldwide. Since publication of the first edition of this book in 1998, the industry has undergone a remarkable expansion, and this has served to underline the need for an accessible source of technical guidance.

This book is unique in providing an overview of the science and technology of the bottled waters industry. The second edition has been strengthened by bringing in a US co-Editor, and the coverage has been thoroughly revised and considerably extended. A new chapter is included on cleaning and disinfection.

The book provides a definitive source of reference for beverage technologists, packaging technologists, analytical chemists, microbiologists and health and safety personnel.

BIOELECTROCHEMISTRY-2005
XVIII International Symposium on Bioelectrochemistry and Bioenergetics (BES)
3rd Spring Meeting: Bioelectrochemistry (ISE)
June 19-24, 2005
Coimbra, Portugal

Bioelectrochemistry includes a broad variety of scholarly approaches leading to a better understanding of all living things at the macroscopic, microscopic/single-cell and nanoscopic/molecular level, producing beneficial applications in medicine, agriculture, industry, and ecology.

The Symposium features all aspects of the highly interdisciplinary area of bioelectrochemistry and bioenergetics, with contributions from the disciplines of biophysics, biotechnology and medical biophysics, on the following themes:

- Electrified interfaces;
- Electron transport in biosystems ;
- Biomembranes and model membranes;
- Bioenergetics and signal transduction;
- Photobioelectrochemistry;
- Biosensors and bioelectronics ;
- Mechanisms of electric and magnetic effects ;
- Biomedical applications ;
- Biocorrosion;
- Biotechnological applications.

In addition to mainstream papers, the programme committee will accept contributions of obvious relevance to the community, which describe important new concepts, underpin understanding of the field or provide important insights into Bioelectrochemistry.

All participants are invited to submit a full paper, which will be subject to the normal peer-review process, before the Symposium, by 31 May 2005.

Manuscripts should be submitted to Prof. Ana Maria Oliveira Brett electronically, through the conference web page.

Please refer to the conference website http://www.bes-ise-2005.uc.pt

THE INTERNATIONAL DAIRY FEDERATION LAUNCHES A NEW WEBSITE
www.fil-idf.org

In support of its efforts to provide the best service to the dairy sector, the IDF has launched a completely revamped website providing detailed information about its core activities, programme of work, events, publications and membership.

The major new element on this new IDF website offers an online consultation of the IDF publications catalogue – Bulletins of IDF, Special Issues, Joint IDF/ISO standards – that can now be searched online. Entering a topic results in a list of publications matching the request. Ordering facilities have also been improved with safe payment online via credit card. Following up of orders is also offered.

This new website is informative for member and non member countries. It includes detailed information about IDF, the advantages that IDF offers, IDF's links with other international organi-

zations whose activities affect the dairy sector.

The site offers the possibility to subscribe to mailing lists to help the visitors keep up-to-date in areas of interest. This easy-to-navigate site also features a new search capability enabling searching right across the site.

For more information, please contact: Marylène Tucci, IDF Communications and Public Affairs Assistant, +32 2 7068644.

ANNUAL WORLD DAIRY SUMMIT INTERNATIONAL DAIRY FEDERATION (IDF)

The Annual World Dairy Summit of the International Dairy Federation was held in Melbourne, Australia from 21-25 November 2004. There were 780 participants from 44 countries and three international organizations were represented.

New IDF President

On 21 November 2004 the International Dairy Federation (IDF) announced that Jim Begg (UK) was elected as its President at the General Assembly in Melbourne, Australia, for a four-year term.

Mr Begg, 54, is currently the Director General of Dairy UK, the recently created organization that represents and promotes the interests of the entire dairy industry in the UK. Prior to the creation of Dairy UK, Mr Begg was Director General of both the Dairy Industry Federation (DIF) (1998-2002) and its successor organization, The Dairy Industry Association (DIAL) (2002-2004). He sits on a number of dairy-related Boards and representative committees, both in the UK and in the EU.

For further information please contact: Edward Hopkin, IDF Director General, +32 2 7339888. Marylène Tucci, IDF Communications and Public Affairs Assistant, +32 2 7068644.

IDF Programme of Work for the Coming Year

Dairy components as ingredients in foods; Animal Health; Farming Practices; Environment; Technology; Hygiene and Safety; Standards of identity; Methods of analysis and sampling; Nutrition and Health; Marketing and Trade Policies; IDF Forum Vancouver 2005.

Farm Management

Terrig Morgan, a dairy farmer from the UK, was appointed to lead the Action Team in Good farming practice, sustainability etc. Progress is expected in the course of 2005.

Development of Dairying

IDF's approach to countries where dairying is in development has been discussed once more. The basic conclusions of a brainstorming session were that it is not justified to maintain this kind of distinction between countries, that IDF should seek to facilitate participation by a larger number of countries by all practical means, that regional meetings may be a productive way for experts from neighboring countries to share experiences and resolve problems in common and that IDF should seek close cooperation with FAO and OIE in these areas.

World Organization for Animal Health (OIE)

IDF's liaison with OIE moves into higher gear with an extended IDF Action Team that brings together all IDF areas of expertise needed to monitor the work of OIE systematically, including infectious disease of cattle, animal welfare and hygiene and safety. IDF's National Committees will be involved in dialogue with their national veterinary authorities who are the official members of OIE.

Conference Highlights

The dairy leaders forum, sponsored by Rabobank, brought together different experiences from Stefan Tomat, Nordmilch (Germany), Miguel Calado, Dean Foods (USA), Stephen O'Rourke, Murray Goulburn (Australia) and Andrew Ferrier, Fonterra (New Zealand)

The International Food Service Industry was the subject of an interesting survey. Changes in European dairy policies were outlined by the head of the European Commission's Dairy Division, Herman Versteylen.

Dairy and heart health emerging benefits of dairy fat and of dairy protein filled two days of the Nutrition Conference. Food Safety, dairy marketing, sustainable cleaning systems, nutri-marketing and future farming systems completed a very extensive three-day programme of conferences.

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Indonesia Joins IDF

IDF celebrated the accession of Indonesia at its General Assembly in Melbourne (Australia) on 21 November 2004. Indonesia, with a population of 210 million, joins the world's two most populous nations China and India and, with Japan, increases IDF's presence in Asia.

This new member brings IDF's family to 42 member countries.

Prof Dr. F.G. Winarno, has been appointed to take the lead and will occupy the position of the first President of the Indonesian National Committee IDF. Prof Winarno was Chairman of the FAO/WHO Codex Alimentarius Commission (1991-1995).

Indonesia becomes a full partner in the dairy sector's voice with international authorities such as the Codex Alimentarius Commission, The Food and Agriculture Organization of the UN (FAO) and the World Organization for Animal Health (OIE). Indonesia will now be a major contributor to the establishment of IDF's programme on Development in Dairying.

Indonesia, also gains access to IDF's worldwide network of 1000 leading experts in all aspects of dairying.

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2004 IDF AWARD GOES TO DR L.K. CREAMER (NZ) AND TO PROF. P. RESMINI (ITALY)

The 2004 IDF Award was granted to Dr Lawrence Kenneth Creamer (New Zealand) and **Professor Pierpaolo Resmini** (Italy) at the IDF Forum on Present and Future Work, during the IDF World Dairy Summit in Melbourne, Australia from 22 to 25 November 2004.

The IDF award recognizes remarkable contributions to progress in international dairying. It is attributed on the basis of a submission by the IDF members, the IDF National Committees. The IDF Award has the form of a piece of art and a medallion with an inscription. In granting the award to these two nominees, the IDF wishes to pay tribute to their extensive contribution to the scientific knowledge in the field of food science, and dairy science in particular.

The first nominee, Dr Creamer joined the New Zealand Dairy Research Institute (currently the Fonterra Research Centre) in 1963 and holds the position of Principal Research Scientist. His career covered a wide area of work including organic chemical reaction mechanisms, ionic complexes in milk, milk payment regimes, casein purification, ca-

sein micelle structure, casein proteolysis, structure and reactions of β-lactoglobulin and κ-casein, chemical modifications and heat treatment of proteins, proteolysis in cheese, association mechanisms in heat and pressure-induced gels, water in cheese and composite gels, and protein polysaccharide interactions. Dr Creamer has been a member of a number of IDF expert working bodies, including cheese rheology, indices of cheese maturation, nitrogen determination and the relationship between protein and nitrogen content of dairy materials. Dr Creamer's contribution to the literature includes approximately 150 refereed primary publications and an equal number of conference presentations. He is a member of the editorial board of the International Dairy Journal and Consulting Editor of the Journal of Dairy Research. Dr Creamer has already received a number of awards including the 1984 Miles-Marschall international ADSA Award "in recognition of exemplary service and leadership in dairy chemistry research and development that has contributed substantially to a better understanding and promotion of the dairy foods industries".

The second nominee, **Professor Resmini** is Full Professor of both Food Science and Dairy Chemistry and Technology in the Department of Food Science and Technology (DISTAM) at the State University of Milan (Italy). In his 35-year research activity, Professor Resmini has developed several original analytical methods for the control of quality and genuineness of milk and dairy products. Some of them have been adopted as official methods in Italy or in the European Union as ISO-IDF International Standards. His studies dealing with chemical and technological aspects of drinking milk processing and the making of typical Italian cheeses, largely adopting electron microscopy and radiochemistry techniques, have contributed to the advance in applied dairy science with a relevant economic impact. He developed other well-known analytical methods for detecting frauds in foods other than dairy products, including the use of common wheat in durum wheat pasta, addition of non-biogenic carbon dioxide in sparkling wine. The original characteristic of Professor Resmini's investigations in the dairy sector was recently recognized with the Hermann-Weigmann medal awarded by the Federal Dairy Research Centre at Kiel (Germany).

For more information, please contact: Monique Lebeau, IDF Administrative Director, +32 2 7339888. Marylène Tucci, IDF Communications and Public Affairs Assistant, +32 2 7068644.

EUROPEAN CONFERENCE: EU-WIDE PROTECTION OF FOODSTUFFS AND AGRICULTURAL PRODUCTS Berlin, 6th to 11th May 2005

Protected Geographical Indication, Protected Designation of Origin and Traditional Speciality Guaranteed will form the focus of a European conference by the Central Marketing Society of German Agriculture. The event will be held in the Estrel Convention Center.

Some 40 European experts from the fields of foodstuffs and agriculture, marketing, law and science will provide comprehensive information on the application of the regulations (EEC) N. 2081/92 (Protected Geographical Indication, Protected Designation of Origin) and N. 2082/92 (Traditional Speciality Guaranteed). The agenda will include marketing-specific factors and legal aspects of the regulations as well as their implementation in the EU member states. In addition, practical examples will be demonstrated and the risks and opportunities of the regulations discussed. The conference, which CMA will be hosting in cooperation with the European Commission, offers participants a platform for exchanging experi-

ences or developing and expanding networks at an international level.

The registration of a food or agricultural product in the EU register on the basis of regulation (EEC) N. 2081/92 guards against improper or misleading use or imitation EU-wide, registration according to regulation (EEC) N. 2082/92 protects the composition of products or the production and/or processing methods. Even if 650 European food and agricultural products have already been entered in the EU register, the potential is far from exhausted. In countries such as France and Italy more than 130 have been protected in each case, in Germany 36, in Austria 12 or in the Czech Republic three.

Registration documents for the conference can be ordered until 21st March 2005 from Euro RSCG ABC Hamburg, Inke Stern, Rödingsmarkt 9, D-20459 Hamburg, Tel. +49 040 43175174, Fax +49 040 43175110, e-mail: inke.stern@eurorscgabc.de.

Since the number of participants is limited, registrations will be treated on a first-come, first-served basis.

2nd INTERNATIONAL CONGRESS SELF-CONTROL AND FOOD SAFETY
October 17 and 18, 2005
Euskalduna Conference Centre
Bilbao, Bizkaia (Spain)

Aims

This conference aims to be a discussion forum on most relevant topics related to the HACCP system and treatment, with a view to achieve a common position amongst all involved food stakeholders, administration, universities, food companies, auditors, and consumers.

Who should attend?

Auditors and certifying bodies; food company quality managers; food law enforcers; consumer associations; consulting companies.

Presentations

A limited number of oral and poster presentations will be accepted on the following topics: monitoring based on quick analytical techniques, pre-requisites and HACCP, HACCP and microcompanies, HACCP implementation problems, HACCP auditing, training and education in HACCP, HACCP documentation, catering and HACCP.

Deadline for receiving papers
30th of June 2005

Language

The official languages of the conference are Spanish and English. Translation services will be provided.

Information: www.kausal.biz

IAFP 2005 TO BE HELD
August 14-17, 2005
Baltimore, Maryland (USA)

The International Association for Food Protection will hold IAFP 2005, the 92nd Annual Meeting from August 14-17, 2005 at the Baltimore Marriott Waterfront Hotel in Baltimore, Maryland. Registration information will be available in January on the Association Web site, www.foodprotection.org. The preliminary program will be available in February 2005.

To provide food safety professionals worldwide with a forum to exchange information on protecting the food supply, IAFP 2005 will feature approximately 26 symposia, 75 technical and 250 poster presentations. During the four-day meeting, the conference will include a business meeting, committee meetings, educational exhibits, an awards banquet, and a number of social events. Four pre-meeting workshops will also be presented.

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GUIDE FOR AUTHORS

ITALIAN JOURNAL OF FOOD SCIENCE - IJFS

1. Manuscript Preparation

(1) Manuscripts must be typed, double-spaced and **two** copies submitted along **with** the computer disk. There should be liberal margins on top, bottom and sides (2.5 cm). English is the official language. Authors who are not fluent in written English must seek help from a person fluent in scientific English. The Assistant Editor reserves the right to make literary corrections and to make suggestions to improve brevity, but **the paper must be revised by a native English speaker** before submission.

Pages and lines on all pages, including those pages for “References” and figure legends, **must be electronically numbered in the left margin**, beginning with number one at the top of the page.

The paper must also be submitted by e-mail or on a digital support (cd-rom or floppy disk). Indicate which word processor was used to generate the file and save the file also in format “Text only”, DCA-RTF or ASCII, if you do not have programs for Macintosh; **graphics, pictures and diagrams must be saved at 300 dpi in TIF, JPEG, EPS or PICT formats** (not included in MsWord documents).

(2) Every paper should be divided under the following headings in this order:

Title. Informative of the content of the article (<50 characters + spaces). Author(s). Initials and Surname, omit professional and official titles. The Institute and address where the research was carried out and the current address of each author should be given as a footnote on the title page.

Abstract. Clearly state the objective of the study, give a concise description of experiment(s), observations, results and conclusions. No references should be cited. DO NOT EXCEED 100 WORDS. An abstract and title in Italian (corresponding to the English) must also be included.

Keywords. Up to six words, in alphabetical order, which describe the document must be given to aid data retrieval and indexing.

Introduction. Review pertinent previous work and cite appropriate references. State the purpose of the investigation.

Materials and Methods. Indicate apparatus, instruments, reagents, etc., giving sufficient detail to allow the work to be repeated.

Results and Conclusions. Results and Conclusions may be presented together or separately. Concisely present results using tables and figures to help justify conclusions (do not present the same information in both forms). Use statistical analysis when appropriate. Unsupported hypotheses should be avoided. Conclusions should point out the significance of the findings and, if possible, relate the new findings to some problem in Food Science and Technology.

Acknowledgments. Acknowledgments of assistance are appropriate provided they are not related to analyses, or other services performed for a fee. Financial support, thanks for assistance, article number or thesis fulfillment may be included.

Units. A list of units particular to the paper may be included.

References. References should be arranged alphabetically, and for the same author should be arranged consecutively by year, typed double-spaced. Each individual citation should begin flush left (no indentation). Refer to attached examples taken from “Style Guide for Research Papers” by the Institute of Food Technologists (Chicago - Illinois - USA). Literature citations in the text should be referred to by name and year in

parentheses (only the initials in capital letters). If there are more than two authors, mention the first author and add *et al.*

(3) Tables should be as few and as simple as possible and include only essential data. Each table must be saved and printed on a separate sheet, and have an Arabic number, e.g. Table 4 NOT Tab. 4. Legends must be self-explanatory and on a separate sheet. Use lower-case letters for footnotes in tables and explain below the table in the order in which they appear in the table.

(4) Figures must be drawn and saved by separate in TIF, JPEG, EPS or PICT formats (300 dpi resolution). They should be drawn so that on 50% reduction, lines, figures and symbols will be clearly legible and not overcrowded. A photocopy of how the figure should appear must be included. Photographs must be unmounted, glossy prints or slides. All figures must be given Arabic numbers, e.g. Fig. 3, in the text and in the final copy only on the back where the title of the paper, the senior author's surname and the top of the illustration must also be marked; for reviewing procedures, do not include this information in the first submitted copies. Legends for figures must be self-explanatory and should be typed on a separate sheet under "Legends to Figures".

(5) Standard Usage, Abbreviations and Units. The Concise Oxford and Webster's English Dictionaries are the references for spelling and hyphenation. Statistics and measurements should always be given in figures, e.g. 10 min, except when the number begins a sentence. When the number does not refer to a unit of measurement it is spelled out unless it is 100 or greater. Abbreviations should be used sparingly, only when long or unwieldy names occur frequently, and never in the title; they should be given at the first mention of the name. International Standard abbreviations should generally be used except where they conflict with current practice or are confusing. For example, 3 mm rather than $3x10^{-3}$ m. Abbreviations should be defined the first time that they are used in the text and they should be used consistently thereafter. Temperatures should be expressed in the Celsius (centigrade) scale. Chemical formulae and solutions must specify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by the Latin binomial (italics) at the first mention. For subsequent use, the generic name should be contracted to a single letter if it is unambiguous.

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Scientific contributions in one of the following forms may be submitted:

Opinions and Reviews - Papers may be sent directly to the Editor-in-Chief who will decide upon publication or articles will be requested directly from the authors by the Editor-in-Chief.

Short Communications and Surveys - They do not need to have the formal organization of a research paper; they will receive priority in publication;

Papers - The paper must follow manuscript preparation.

Short Communications, Surveys and Papers will be subjected to critical review by the referees. Upon receiving papers from authors, the Advisory Board with the Editor-in-Chief will select papers in relationship to innovation and originality and send copies to the referees. A letter stating that the paper has been accepted for refereeing will be sent to the authors. Papers needing revision will be returned to the author, and the author must return the revised manuscript to the Editor-in-Chief, otherwise the paper will be considered as withdrawn. Papers not suitable for publication will be returned to the author with a statement of reasons for rejection.

3. Editorial Policy

Referees may not be from the same institution as the author. Referees should make their comments and questions in detail and return the paper to the Editor-in-Chief as

soon as possible, usually within 4 weeks. The identity and the report of the referees are made known to the Editor-in-Chief, but only the anonymous report is routinely sent to the author. If all referees recommend acceptance or rejection, the decision stands. If the opinions of the referees tie, the Editor-in-Chief has the freedom to decide upon acceptance or rejection of the paper. Manuscripts will be edited in the order received and accepted papers will be published as closely as possible in this order. A letter announcing the issue of publication will be sent to the author after the manuscript has been accepted by the Editor-in-Chief. Each paper is accepted with the understanding that it is the sole document under active consideration for publication covering the work reported (it has NOT been previously published, accepted or submitted for publication elsewhere). Upon acceptance of the paper for publication, the author agrees to pay the page charges as published on the first page of each issue. Authors take full responsibility for all opinions stated in their papers and published in this journal.

4. Mailing Instructions

Papers for publication and communications regarding editorial matters should be sent to:
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The proofs will be sent to the corresponding author as a PDF file by e-mail only. A hard copy will be sent by mail only if the author makes this reguest when the paper is accepted for publication.
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EXAMPLES of use in a Reference list are given below. The bold-faced parenthetical type of citation above the example is indicated ONLY for information and is NOT to be included in the reference list.

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