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RESEARCH ARTICLE

New Clones and Old Varieties: Quality of Sicilian Hillside Apple Cultivation

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Abstract:

Aims:

The aim of this study is to evaluate the qualitative characteristics of the new clones according to the Mediterranean hillside growing environment and, at the same time, to highlight the qualitative peculiarities of the old varieties in order to avoid genetic loss.

Introduction:

Several apple varieties are constantly selected for improved quality traits and introduced for cultivation and marketing in addition to a few traditional and affirmed varieties. On the other side, local genotype and ancient varieties are still valorised due to the request of a niche market.

Methods:

We have studied the physico-chemical quality and the sensory traits of the fruit obtained in this particular environment.

Results:

Our study reveals a qualitative response to the environment in a genotype-dependent manner. As expected, the physico-chemical characteristics favour the new clones.

Conclusion:

Both old varieties and new clones of apple fruit, grown in the Mediterranean area, turned out to be of high quality. Nevertheless, results revealed the better characteristics of new clones for commercialization in large-scale supply chain.

Keywords: Apple, Germplasm, Varietal renewal, Genetic loss, Biodiversity, Sensory analyses.

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1. INTRODUCTION

The near-extinction of many old genotypes in the last decade has led to the loss of a part of horticultural biodiversity. Some of these cultivars are characterised by specific aromas and flavours, are more easily adapted to different climatic conditions, and have quality characteristics that are absent in new cultivars [1, 2]. Recent studies have highlighted the importance of characterization of germplasm species for the enhancement of local genetic resources [3, 4]. In fact, in recent years, we are witnessing a renewed interest in the revaluation and enhancement of old varieties that have interesting quality attributes [5, 6]. At present, old varieties directed at specific markets [7] are a source of useful genes for breeding programmes which, due to their resistance, could ensure organic or low impact cultivation regimes [8]. Nevertheless, some fruit cultivars grown all over the world have become predominant, although older cultivars have specific quality characteristics, requiring fewer chemical inputs, significantly reducing their release into the air, water, and soil, and minimising their negative impacts on human health and the environment [9]. However, the old cultivars, although well adapted to local conditions, were considered obsolete [7]. The demands of large-scale distribution increasingly exclude local varieties and ecotypes. Progressive substitution with modern cultivars is aimed to optimise the yield and the market requirements [10]. In addition, the use of new biotechnological tools should speed up the development of new varieties, saving time and reducing the workloads [11]. This is particularly true for the apple cultivation (*Malus domestica* Borkh.), which are widely grown in Italy. Several apple clones are constantly being selected for

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improved quality traits and introduced for cultivation and marketing, in addition to some traditional, established varieties [8]. On the other hand, local genotypes and old varieties are still valued on the demand of a niche market. In Sicily, both old genotypes and new clones coexist which are mainly located in the organic orchards of the inland areas, in particular in the Etna district [2, 12] and the Madonie Mountains [5, 13]. This is a hillside apple-growing with interesting organoleptic characteristics of cultivated fruit that differ from the rest of Italy [14]. These environments are located above 800 m a.s.l. but are characterised by a typical Mediterranean climate: prolonged frosts that rarely occur in winter, while in summer, the temperature is around 28 - 30 °C. There is usually around 700 - 800 mm of annual rainfall, mainly distributed over the autumn/winter period.

Some of the apple cultivars (both old varieties and new clones) grown in this environment have been studied, showing a delicious taste, good firmness and colour, and long shelf-life [15]. However, the old varieties are more resistant to diseases than the new varieties [16 - 18] and have shown quality traits [8, 19]. For example, a very common disease in apple fruits is the bitter pit, commonly associated with low Ca and high Mg concentration in the tissue. In this context, old cultivars represent a very important reserve for the fruit heritage because they maintain a wide varietal range. Moreover, some quality characteristics are exploited for genetic improvement programmes, including sustainable development and biodiversity conservation [20]. On the other hand, the main objective of breeding programmes is to bring a high-quality product to the market. In particular, shape, colour, crispness, and juiciness are considered essential quality characteristics for success [21]. These parameters are particularly demanded by consumers and replace conventional parameters, such as size, skin colour, and sweetness.

This study aims to analyse the quality characteristics of 5 old apple genotypes and 4 new apple clones grown in a Mediterranean hill climate. In both cases, it is useful to define the physico-chemical quality and the sensory traits of the fruits obtained in this particular environment in order to evaluate their commercial potential and the possibility to save and enhance the old ones and promote the new ones.

2. MATERIALS AND METHODS

One hundred fruits were harvested in 2017 in an experimental field located in Caltavuturo (Sicily, Italy) (37°49' N and 13°53' E) at about 805 m.a.s.l. All genotypes were replicated in three plants, with tree spacing of 1.5 m and 4 m between rows, grafted on M26 rootstock, spindle-trained, and subjected to the ordinary cultural treatments of the organic growing regime. Harvesting was carried out at commercial maturity, and the starch iodine test was used as a maturity index.

In particular, 5 old germplasm varieties (*Cannamelo, San Giuseppe, Virchiata, Turco, and Faccia Rossa*) and 4 new clones belonging to the "List of recommended fruit varieties" programme (*Majesti, Sinfonia, Shinano Gold and Luresweet Redlove*®), were collected. Ten fruits per plant were harvested for each variety. Finally, the fruits were transported to the laboratories of the Department of Agricultural, Food, and Forestry Sciences (SAAF) for analysis.

2.1. Physico-Chemical Analysis

Fruit Weight (FW) was determined using a two-decimal precision digital scale (GIbertini, Italy) and expressed in grams. Transversal and longitudinal diameter (TD and LD, respectively) were determined with a TR53307 digital caliper (Turoni, Forlì, Italy) and expressed in millimetres. The percentage of cover colour (CC) was calculated by means of a pre-calibrated Minolta Chroma Meter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Tokyo, Japan). The colour samples were measured against Hunter values: brightness ($L*$ value) from 0 (black) to 100 (white), green ($a*$ value) from -a (green) to +a (red) and yellow ($b*$ value) from -b (blue colour intensity) to +b (yellow colour intensity) [22]. Firmness (F) was determined by means of a TR5325 digital penetrometer (Turoni, Forlì Italia) with an 8 mm diameter plunger and expressed as $kg{\cdot}cm^{-2}$. After the apple juice extraction with a centrifugal squeezer (Ariete, Italy), the Soluble Solids Content (SSC), expressed as °Brix, was measured by means of an ATAGO digital optical refractometer (Atago Co, Ltd., Tokyo, Japan), while the Titratable Acidity (TA), expressed as malic acid ($g{\cdot}l^{-1}$), and the SSC/TA ratio, were measured with a Crison Compact titrator pH-meter (Crison Instruments, SA, Barcelona, Spain).

2.2. Starch Content

The apples were cut length-wise and the top was immersed in an iodinated solution (Lugol reagent: 40g KI + 10g L^{-1} H_2O) for about 60 seconds. The fruit was then removed from the solution and allowed to air dry for a further 60 seconds. The surface undergoes a colour change, indicating the starch content. This is compared to a reference chart (Eurofru, Ctifl, France) with a scale from 0 (highest starch content) to 6 (no starch content).

The starch state was analysed by Lugol's iodine, compared with reference charts (Eurofru, CTIFL, France), and expressed on a 10-point scale (0 = maximum starch content, 6 = no starch).

2.3. UPOV Analysis

Biometric data were also determined using a specific descriptor list adapted from UPOV (International Union for the Protection of New Varieties of Plants) guidelines [23] to discriminate the different cultivars. In particular, the following descriptors were evaluated: general shape (GS), ribbing (R), stem cavity depth (DS), stem cavity thickness (TS), ocular basin depth (DB), ocular basin thickness (TB), stem length (LS), stem thickness (TS), peel colour (CP), peel cover colour (CP), cover colour type (TC) and flesh colour (CF).

2.4. Sensory Analysis

Sensory profiling (UNI 10957) [24] was performed on a subsample of 10 fruits per cultivar by a panel of 10 judges (50% male and 50% female). All panellists were trained in the sensory evaluation of fruits, according to other studies [25]. Twenty different attributes were generated on the basis of the citation frequency (> 60%), as listed below: appearance (A), peel colour (PC), flesh colour (FC), apple odour (AO), herbaceous odour (HO), almond odour (AO), off-odour (OO), sweetness (S), bitterness (B), acidity (AC), astringency (AS), crunchiness (C), mealiness (M), juiciness (J), apple flavour (AF), herbaceous flavour (HF), honey flavour (HOF), almond flavour (ALF), off-flavour (OF) and overall assessment (OA). The judges evaluated the intensity of each attribute by assigning a score between 1 (absence of the descriptor) and 9 (high presence of the descriptor).

2.5. Chemical Composition

Complex carbohydrates have a significantly more elaborate biochemical structure than other carbohydrates (simple sugars with a simpler structure, determined with the Fehling reagent). The carbohydrate content (TSG), either free or present in polysaccharides, was obtained with the anthrone method reported in Loewus studies [26]. Carbohydrates were first hydrolysed into simple sugars using dilute hydrochloric acid. In a hot acidic medium, glucose is dehydrated to hydroxymethylfurfural that with anthrone forms a green-coloured product with an absorption maximum at 630 nm.

Ash content was determined through the procedure described in the Association of Official Analytical Chemists (AOAC) [27 - 31]. The contents of Ca, Fe, Mg, K, and Na were determined using atomic absorption spectroscopy following wet mineralization. The determination of vitamins (B1, B5, and E) was determined by HPLC after extraction of the lipophilic component from pulp samples through AOAC procedures. The riboflavin (vitamin B2) was extracted in an autoclave with a solution of diluted H_2SO_4 and later, after enzymatic treatment, vitamin B2 (mg%) was quantified was determined through HPLC (for the fluorescent spectra). For the ascorbic acid (vitamin C) determination, the dried methanolic extract (100 mg) was extracted with 10 mL of 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 mL) was mixed with 9 mL of 2.6–dichlorophenolindophenol, and the absorbance was measured within 30 min at 515 nm against a blank. Ascorbic acid was calculated based on the calibration curve of authentic L-ascorbic acid (0.02 – 0.12 mg mL−1) [32].

Moisture Content (MC), TSG content, and ASH content were expressed as g%, while minerals and vitamins were expressed as mg%. All measurements were done in three replicates.

2.6. Statistical Analysis

The physico-chemical and sensory data were tested for differences between the cultivars using the one-way analysis of variance (ANOVA: general linear model) using XLSTAT software version 9.0 (Addinsoft, Paris, France). The differences between cultivars were tested, followed by Tukey's multiple range test for $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physico-Chemical Analysis

Our data showed significant differences between the different apples in a genotype-dependent manner. In particular, Table **1** shows the differences in physico-chemical characteristics.

As expected, the new clones showed a higher weight than the old varieties. Probably the detected weight was influenced by the thinning of the fruits, which was done in a single step. For this reason, the fruits might be slightly undersized [33]. According to the commercial classification in size classes (EC Reg. n.85/2004) [34], considering both transverse diameter and weight, all observed genotypes would be included in the extra category (Table **2**). Similar data were observed by Lo Bianco and Farina [8].

Firmness showed very high values in all observed fruits, showing a good aptitude for cold storage. Old varieties ranged from 3.12 (*Faccia Rossa*) to 4.68 (*San Giuseppe*). The new clones varied from 2.83 (*Sinfonia*) to 4.46 (*Luresweet Redlove®*). The old varieties have values above 3.5 in three cases because they are probably derived from old spontaneous hybridisation with *Cydonia oblonga* [8, 35].

Table 1. Fruit weight (FW), longitudinal diameter (LD), transversal diameter (TD), firmness (F) and juice (J) content of the observed old varieties and new clones. Data are means ± standard deviation SD (n = 30). Different letters along each column indicate significant differences among genotypes at *p* ≤ 0.05 using Tukey's test.

Genotypes		FW (g)		LD (mm)		TD (mm)		F (kg cm^{-2})		J (g/100 g of flesh)	
Old Varieties	*Cannamelo*	118.05 ± 20.29	c	55.65 ± 3	d	65.56 ± 4.41	bc	3.13 ± 0.37	d	60.31 ± 1.5	bc
	Faccia Rossa	125.69 ± 28.46	bc	56.78 ± 7.5	cd	64.19 ± 4.59	cd	3.12 ± 0.43	d	55.27 ± 9.31	c
	San Giuseppe	99.41 ± 18.12	c	50.6 ± 4.32	de	61.4 ± 4.39	d	4.68 ± 0.29	a	56.6 ± 5.66	c
	Turco	101.36 ± 13.88	c	55.86 ± 0.72	d	60.33 ± 1.78	d	3.68 ± 0.29	c	70.15 ± 0.16	a
	Virchiata	110.84 ± 14.29	c	53.49 ± 3.24	de	63.4 ± 3.06	cd	4.07 ± 0.28	b	53.86 ± 9.05	d
New clones	*Majesti*	153.48 ± 19.46	b	68.58 ± 4.11	b	67.11 ± 3.28	bc	2.96 ± 0.34	d	62.5 ± 11.4	b
	Luresweet Redlove®	148.77 ± 19.03	b	59.82 ± 4.6	c	65.13 ± 4.97	cd	4.46 ± 0.39	a	61.33 ± 17.92	b
	Shinano Gold	158.22 ± 15.42	b	62.02 ± 6.76	c	69.08 ± 6.31	b	3.12 ± 0.32	d	67.45 ± 3.04	a
	Sinfonia	219.12 ± 13.14	a	75.72 ± 3.64	a	78.2 ± 4.27	a	2.83 ± 0.36	d	69.98 ± 0.96	a

Table 2. Commercial classification, according to EC Reg. n.85/2004 concerning the apples commercialization

Minimum calibre (by transversal diameter) for commercial apples in EU			
	Extra	**I Class**	**II Class**
Large size fruits	65 mm	60 mm	60 mm
Others	60 mm	55 mm	50 mm
Minimum calibre (by weight) for commercial apples in EU			
	EXTRA	I CLASS	II CLASS
Large size fruits	110 g	90 mm	90 mm
Others	90 g	80 g	70 g

Concerning colorimetric analyses (Fig. **1** and Table **3**), for the old genotypes, the highest percentage of cover colour (CC%) was recorded in *Cannamelo, Turco*, *Majesti* and *Luresweet Redlove®* which was almost uniformly red, while the lowest was recorded in *Shinano Gold,* being yellow apple. For brightness (L^*), the highest value was recorded in *Faccia Rossa, San Giuseppe, Shinano Gold* and *Sinfonia*. For the parameter a^* (red = +100, green= -100), the highest value was recorded in the new clones (*Majesti*, *Luresweet Redlove®* and *Sinfonia*). For b^* (yellow = +100, blue= -100). *Faccia Rossa*, *Turco, Shinano Gold,* and *Sinfonia* showed the highest value. The results of colorimetric analyses showing that fruits from the new clones are potentially more appreciable for the fresh consumption market could be due to good visual characteristics, which correspond to the maximum brightness index, in agreement with Ivascu *et al.* [36] and Iglesias *et al.* [37], according to whom consumers are strongly attracted to the more colourful fruits, as well as the *San Giuseppe* among the old varieties (Fig. **1**).

As for the juiciness analysed in a sample of 100 g of fruit (Fig. **2**), all the new clones showed high values, whereas only *Faccia Rossa* and *San Giuseppe* reached the lowest values.

With regard to SSC, all the observed genotypes show very high values. New clones varied from 12.38 to 16.66 Brix°, whereas old varieties varied from 13.31 to 17.16 Brix°. In particular, *Shinano Gold* and *Luresweet Redlove®* have the highest content matched only by *Cannamelo* among the older varieties. As for TA, the differences are also large within each genotype group. The values range from 4.1 (*Turco*) to 7.44 g of malic acid per litre (*Cannamelo)* in old varieties, while in the new clones, they vary between 4.46 (*Shinano Gold)* and 8.93 (*Luresweet Redlove®)*. The SSC/TA ratio is strongly influenced by the high variability of the two chemical parameters and, in particular, of TA. The genotypes can be grouped into three subgroups i) value slightly lower than 2 (*Virchiata, Majesti, Luresweeet Redlove®*), ii) values higher than 2 (*Cannamelo, San Giuseppe* and *Sinfonia*), and iii) values higher than 3 (*Faccia Rossa, Turco* and *Shinano Gold*).

Table 3. Lightness (L^*), greenness (a^*), yellowness (b^*) and cover colour (%) of the observed old varieties and new clones. Data are means ± standard deviation SD (n = 30). Different letters along each column indicate significant differences among genotypes at $p \leq 0.05$ using Tukey's test.

Genotypes		***L****	***a****	***b****	**CC%**
Old varieties	*Cannamelo*	37.17 ± 2.02 d	26.20 ± 2.42 cd	16.02 ± 2.17 c	95.00 ± 5.53 a
	Faccia Rossa	43.00 ± 3.30 bc	28.85 ± 3.59 bc	18.03 ± 2.39 bc	71.67 ± 11.69 c
	San Giuseppe	44.54 ± 3.64 bc	29.12 ± 2.96 bc	17.96 ± 3.35 bc	66.67 ± 8.16 c
	Turco	37.70 ± 2.98 d	22.93 ± 3.67 d	12.79 ± 2.54 d	95.00 ± 4.37 a
	Virchiata	42.82 ± 2.27 bc	30.77 ± 3.34 b	18.88 ± 1.86 bc	76.00 ± 14.78 bc
New clones	*Majesti*	37.55 ± 2.09 d	36.08 ± 2.39 a	16.21 ± 1.90 c	84.17 ± 9.70 b
	Luresweet Redlove®	40.84 ± 3.59 cd	36.04 ± 3.54 a	16.85 ± 2.45 c	95.00 ± 7.56 a
	Shinano Gold	72.65 ± 2.98 a	-7.74 ± 6.91 e	47.91 ± 3.60 a	6.67 ± 8.75 d
	Sinfonia	45.32 ± 3.40 b	36.40 ± 2.64 a	21.75 ± 1.31 b	71.67 ± 10.33 c

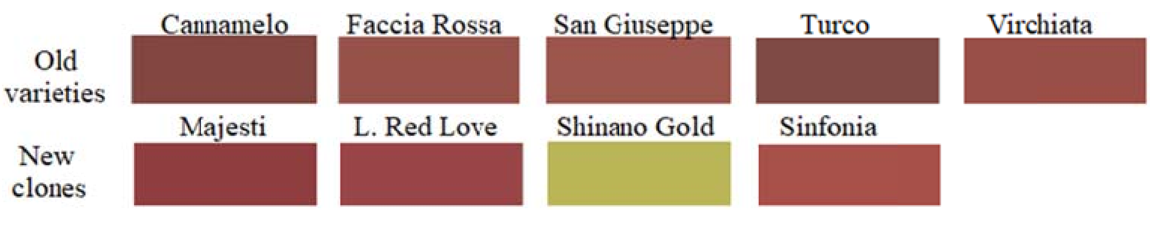

Fig. (1). Colour table obtained from the results of the $L^*a^*b^*$ colour recorded in the CIEL*a*b* colour space and converted to the red/green/blue (RGB) scale through the www.e-paint.co website.

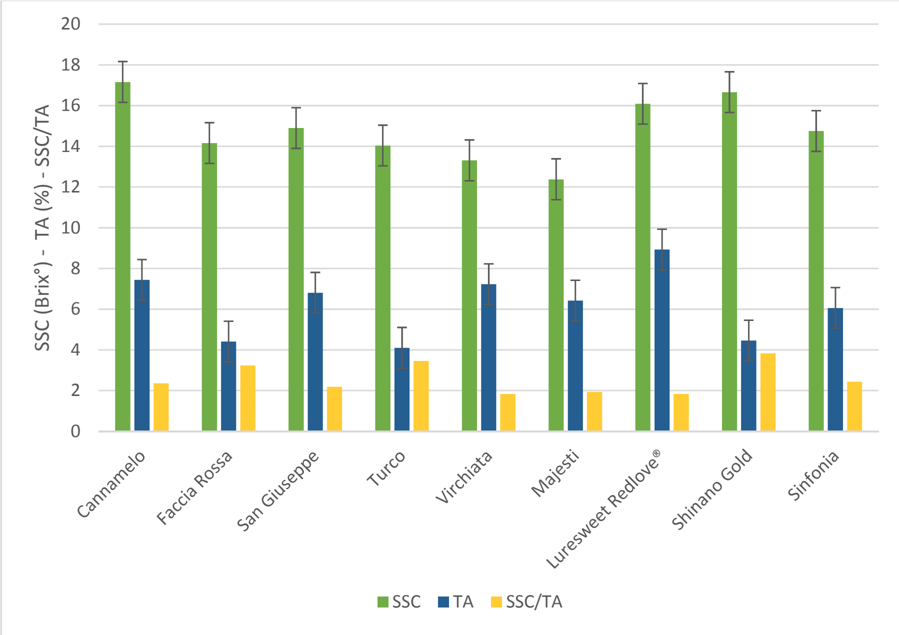

Fig. (2). Soluble solids content (SSC), titratable acidity (TA), and their ratio (SSC/TA) of the observed old varieties and new clones. Values indicate the means, and the bars indicate the standard deviations of the replicates (n = 30).

3.2. Starch Content

Considering the data measured with Lugol's test for starch content, the values vary on a scale between 0.6 and 1.0 (data not shown), indicating a fairly similar ripening stage. This small variability is closely related to the genotype and, although there are no specific tables for old varieties and new clones, we have tried to conform to what we found in the literature [38].

3.3. UPOV Analysis

Finally, the fruits of each variety were subjected to analyses regulated by the UPOV system. These analyses allowed the attribution of an adjective to each fruit, which was visually detected (Table **4**).

3.4. Sensory Analyses

According to the results of the statistical analysis, the most appreciated cultivars were *Faccia Rossa* and *Cannamelo*, which presented appreciable characteristics of flesh crispness, skin and flesh colour, apple flavour and especially sweet taste, as shown in Fig. (**3a**) (old cultivars),. On the contrary, *Virchiata* and *San Giuseppe* showed less appreciable characteristics, showing higher values of honey taste, almond smell, and acidity, which are rather negative characteristics for the sensory analysis of apples. In addition, low sweetness values were recorded.

Table 4. UPOV descriptors applied to the observed genotypes. General shape (GS), Ribbing (RI), Depth of stalk cavity (DS), Width of stalk cavity (WS), Depth of eye basin (DB), Width of eye basin (WB), Length of the stalk (LS), Thickness of stalk (TS), Skin colour (SC), Hue cover colour (HC), Cover colour typology (CT), Flesh colour (FC).

CULTIVAR	GS	RI	DS	WS	DB	WB	LS	TS	SC	HC	CT	FC
Cannamelo	obloid	absent	medium	narrow	medium	medium	very short	medium	green-yellow	red-brown	large strips	cream
Faccia Rossa	obloid	absent	medium	medium	medium	broad	long	medium	green-yellow	red-brown	flushed and mottled	cream
San Giuseppe	obloid	absent	deep	medium	shallow	broad	very short	medium	green-yellow	red-brown	flushed and mottled	white
Turco	globose	absent	medium	medium	medium	medium	long	medium	green-yellow	red-brown	uniform	white
Virchiata	globose	absent	high	broad	shallow	broad	short	thin	green-yellow	red	large strips	green
Majesti	conic	absent	deep	medium	shallow	medium	long	medium	white-yellow	red-brown	large strips	white
Luresweet Redlove®	conic	absent	medium	medium	shallow	medium	long	medium	green	red	only solid flush	reddish
Shinano Gold	conic	absent	shallow	narrow	deep	medium	medium	medium	yellow	orange-red	only solid flush	cream
Sinfonia	conic	absent	deep	medium	medium	medium	medium	medium	green-yellow	red	weakly defined flush with large strips	cream

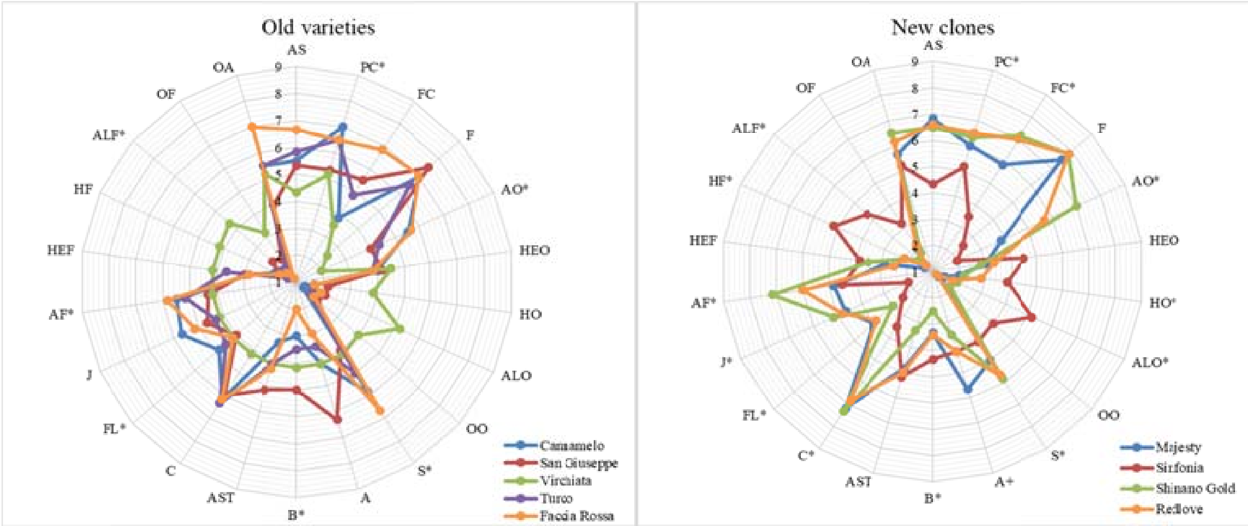

Fig. (3). Sensory evaluation of the observed old varieties and new clones; Descriptors legend: As: Aspect, PC: Peel Colour, FC: Flesh Colour, F: Firmness, AO: Apple Odour, HEO: Herbal Odour, HO: Honey Odour, ALO: Almond Odour, OO: Off Odour, S: Sweetness, A: Acid, B: Bitterness, AST: Astringency, C: Crispness, FL: Flouriness, J: Juiciness, AF: Apple Flavour, HEF: Herbal Flavour, HF: Honey Flavour, ALF: Almond Flavour, OF: Off Flavour, OA: Overall Acceptability; The * symbol indicates significant differences found by statistical analysis ($p \geq 0.05$).

With regard to the new clones (Fig. **3b**), the highest percentage of positive evaluations occurred in *Shinano Gold*, which presented good characteristics in terms of crispness, apple taste, and smell, firmness and sweetness. In addition, positive values were found concerning the acid content of the fruit, which was only perceived in small quantities, and concerning the bitter taste, which was not perceived. An interesting result was found for the variety *Luresweet Redlove®* due to its particular flesh colour.

3.5. Chemical Composition

The moisture content (MC - Table **5**) is highest in *Turco, Majesti*, *Faccia Rossa*, *Shinano Gold* and *Sinfonia,* which were also the juiciest. It is obvious that the higher the MC of fruit, the fresher it appears [39]. Moisture content is an important factor as it can influence the texture and overall acceptability, but on the other hand, it can directly influence the shelf-life of the fruits. In fact, moisture loss during the long-term storage of apples causes a direct economic loss due to a decrease in saleable weight. The highest values of carbohydrate content (TSG) were observed in *San Giuseppe* and *Virchiata*, while the ASH content is very variable.

The results concerning the analysis of proximate compounds (Table **6**) showed significant genotype-dependent differences, according to other studies [40, 41]. In all observed genotypes, Mg and Na values are in line with those found on the USDA nutrient database [40]; Ca and Fe have higher values and, K and Na have lower values. In particular, with regard to the Ca content, *Majesti* (new clones) has the highest value while, among the old varieties, *Virchiata* has the highest Fe value. The Mg content is similar between new and old varieties, but the concentration is higher in the old varieties. In terms of K content, the old varieties show the highest values and, among them, *Turco* has relevant content. On the contrary, in terms of Na content, the new clones and, in particular, *Sinfonia* and *Majesti*, have the highest values.

Table 5. Moisture content (MC), carbohydrate content (TSG), and ASH content of the observed old varieties and new clones. Data are means ± standard deviation SD (n = 30). Different letters along each column indicate significant differences among genotypes at *p* ≤ 0.05 using Tukey's test.

Genotypes	MC (g%)				TSG (g%)				ASH (g%)			
Old varieties	-	-	-	-	-	-	-	-	-	-	-	-
Cannamelo	81.52	±	0.2	c	10.63	±	0.36	ab	22.00	±	0.2	bc
Faccia Rossa	83.15	±	0.2	b	8.24	±	0.45	b	28.00	±	0.1	c
San Giuseppe	80.90	±	0.8	c	14.74	±	1.04	a	1.00	±	0.1	b
Turco	84.73	±	1.6	a	9.01	±	1.26	b	7.00	±	0	c
Virchiata	83.05	±	0.2	b	15.04	±	0.5	a	5.00	±	0	b
New Clones	-	-	-	-	-	-	-	-	-	-	-	-
Majesti	85.46	±	0.9	a	6.50	±	1.1	c	24.00	±	0.1	a
Luresweet Redlove®	83.56	±	0.6	b	9.93	±	0.34	b	35.00	±	0.2	a

(Table 5) contd.....

Genotypes	MC (g%)				TSG (g%)				ASH (g%)			
Shinano Gold	85.13	±	0.1	a	8.89	±	1.38	b	9.00	±	0.1	b
Sinfonia	85.26	±	0.1	a	9.49	±	0.7	b	4.00	±	0.1	a

Table 6. Calcium (Ca), Iron (Fe), Magnesium (mg), Potassium (K), and Sodium (Na) of the observed old varieties and new clones. Data are means ± standard deviation SD (n = 30). Different letters along each column indicate significant differences among genotypes at *p* ≤ 0.05 using Tukey's test

Genotypes	Ca (mg%)				Fe (mg%)				Mg (mg%)				K (mg%)				Na (mg%)			
Old varieties	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cannamelo	27.24	±	0.02	ab	0.17	±	0.18	b	0.41	±	0.04	b	65.05	±	0.07	c	12.20	±	0.05	c
Faccia Rossa	27.30	±	0.03	a	0.18	±	0.17	b	0.55	±	0.10	a	78.21	±	0.11	ab	12.31	±	0.07	c
San Giuseppe	26.14	±	0.03	b	0.19	±	0.16	b	0.49	±	0.04	ab	55.33	±	0.08	d	12.21	±	0.04	c
Turco	26.33	±	0.03	b	0.15	±	0.20	b	0.48	±	0.03	ab	84.40	±	0.10	a	9.40	±	0.40	d
Virchiata	24.01	±	0.08	c	1.62	±	1.27	a	0.51	±	0.06	a	75.13	±	0.07	b	10.54	±	0.13	cd
New Clones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Majesti	28.54	±	0.04	a	0.20	±	0.15	b	0.39	±	0.06	b	42.32	±	0.07	f	19.30	±	0.15	a
Luresweet Redlove®	24.13	±	0.01	c	0.22	±	0.13	b	0.42	±	0.25	ab	41.41	±	0.10	f	17.47	±	0.70	b
Shinano Gold	14.21	±	0.10	e	0.21	±	0.14	b	0.37	±	0.08	b	45.74	±	0.12	e	17.72	±	0.06	b
Sinfonia	21.20	±	0.03	d	0.19	±	0.16	b	0.39	±	0.06	b	48.33	±	0.09	de	20.21	±	0.12	a

Concerning the vitamin content (Table **7**), the results show that all the analysed groups have a great variability between all genotypes. In particular, the old varieties generally have higher values than the new clones. Among the new clones, *Sinfonia* has similar values to the old varieties and, in particular, *Virchiata*, *Cannamelo,* and *Turco*. The activity of vitamins, known as "phytonutrients," is commonly included in the anti-oxidant properties of many fruits or vegetables. Thiamine, (vitamin B1) is one of the water-soluble vitamins and should be taken regularly through the diet. Vitamin B1 contributes to the important process of converting glucose into energy. Vitamin B1 deficiency can also lead to heart disease, neurological disorders, dilated pupils, and hypersensitivity of the spine. Vitamin B5 exists in food in bound and free forms and is involved in many biological functions. Its deficiency is associated with metabolic and energy disorders in humans [42]. Epidemiological studies show that diets rich in fruit and vegetable with vitamin C are associated with a lower risk of cardiovascular disease, stroke, cancer, and increased longevity [43 - 45]. Furthermore, plant-based foods contributes vitamin E to the human diet, which, among other essential nutrients, may act against free radicals [46, 47]. Indeed, scientific evidence shows that diets based on vitamin E-rich plant products, such as the traditional Mediterranean diet, could prevent coronary heart disease [48] and cancer (World Cancer Research Fund) [49]. Riboflavin (vitamin B2) is important for the prevention of cardiovascular disease, cancer, incipient diabetic nephropathy, and retinopathy and, this value is better in new cultivars, whereas old cultivars have the lowest value.

Table 7. Thiamine, Vitamin B5, Vitamin C, Vitamin E, and Vitamin B2 of the observed old varieties and new clones. Data are means ± standard deviation SD (n = 30). Different letters along each column indicate significant differences among genotypes at *p* ≤ 0.05 using Tukey's test.

Genotypes	Thiamine (mg%)				Vitamin B5 (mg%)				Vitamin C (mg%)				Vitamin E (mg%)				Vitamin B2 (mg%)				
Old varieties	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cannamelo	17.21	±	0.1	b	85.21	±	0.04	ab	8.24	±	0.14	a	15.11	±	0.1	b	13.75	±	0.2	bc	-
Faccia Rossa	18.32	±	0.12	b	92.33	±	0.02	a	8.69	±	0.18	a	20.3	±	0.1	a	15.34	±	0.1	b	-
San Giuseppe	18.25	±	0.4	b	79.12	±	0.01	b	7.89	±	0.12	ab	17.42	±	0.02	b	12.45	±	0.6	c	-
Turco	15.11	±	0.07	b	78.75	±	0.05	b	7.24	±	0.09	ab	21.89	±	0.04	a	22.81	±	0.12	a	-
Virchiata	19.41	±	0.4	a	81.12	±	0.06	ab	7.47	±	0.11	ab	21.03	±	0.02	a	23.7	±	0.14	a	-
New Clones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Majesti	15.52	±	0.1	b	61.45	±	0.04	c	7.51	±	0.04	ab	11.44	±	0.04	b	15.41	±	0.1	c	-
Luresweet Redlove®	10.21	±	0.8	b	54.31	±	0.01	d	7.21	±	0.07	ab	10.75	±	0.06	b	18.89	±	0.2	b	-
Sinfonia	18.41	±	0.03	b	50.7	±	0.04	d	7.02	±	0.08	b	15.69	±	0.05	a	19.78	±	0.1	a	-
Shinano Gold	15	±	0.12	b	55.24	±	0.03	d	7.4	±	0.16	ab	12.85	±	0.09	b	18.69	±	0.2	b	-

CONCLUSION

Both old and new clones, grown in the Mediterranean hill climate, expressed a high level of fruit quality. As consumers are strongly attracted to the more colourful, sweeter, and vitamin-rich fruits, the new clones are potentially more appealing to the fresh fruit market. Indeed, from the obtained results and from analytical and sensory analyses, it is clear that varietal renewal represents an important means for the commercial offer of apples. On the other hand, the old genotypes have reached interesting quality characteristics, not only for the niche market.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

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REFERENCES

[1] Bounous G, Beccaro G L, Mellano M G. Antiche cultivar di melo in Piemonte. 2006.

[2] Guarrera N, Sperlinga E, Passerini A, Maccarone E. Evaluation of the aromatic and polyphenolic composition of cola and cola gelato apples grown in the area of the Etna volcano. Ital J Food Sci 2008; 20(3): 351-64.

[3] Farina V, Gianguzzi G, Mazzaglia M. Fruit Quality Traits of Six Ancient Apple (Malus domestica Borkh) Cultivars Grown in the Mediterranean Area. Int J Fruit Sci 2016; 16(3): 275-83. [http://dx.doi.org/10.1080/15538362.2015.1102675]

[4] Gentile A, Cartabellotta D, Spartà G, Continella G. Produzioni frutticole autoctone nel massiccio etneo (Sicilia). Inf Agrar 2006; 62: 24-7.

[5] Farina V, Mossad A. Yield and fruit quality characterization of eight old sicilian apple cultivars. Int J Fruit Sci 2011; 11(3): 264-75. [http://dx.doi.org/10.1080/15538362.2011.608298]

[6] Farina V, Barone F, Mazzaglia A, Lanza CM. Evaluation of fruit quality in loquat using both chemical and sensory analyses. Acta Hortic. 887: 345-9. [http://dx.doi.org/10.17660/actahortic.2011.887.59]

[7] Farina V, Gianguzzi G, Mazzaglia A. Fruit quality evaluation of affirmed and local loquat (Eriobotrya japonica Lindl) cultivars using instrumental and sensory analyses. Fruits 2016; 71(2): 105-13. [http://dx.doi.org/10.1051/fruits/2015053]

[8] Lo Bianco R, Farina V. Fruit quality of Sicilian, commercially affirmed, and newly selected apple varieties: Potential resources for genetic improvement and specialty markets in apples. Nutrition, Consumption and Health Novapublischer 2012; pp. 193-203.

[9] Bartolini S, Viti R, Ducci E. Local fruit varieties for sustainable cultivations: Pomological, nutraceutical and sensory characterization. Agrochimica 2015; 59(4): 281-94.

[10] Testa R, Migliore G, Schifani G, Tinebra I, Farina V. Chemical–physical, sensory analyses and consumers' quality perception of local *vs*. Imported loquat fruits: A sustainable development perspective. Agronomy (Basel) 2020; 10(6): 870. [http://dx.doi.org/10.3390/agronomy10060870]

[11] Sansavini S, Belfanti E, Costa F, Donati F. European apple breeding programs turn to biotechnology. Chron Hortic 2005; 45(2): 16-9.

[12] Continella G, Catalano M, Cicala A, La Malfa S, Caruso M, Domina F. Domina. Il germoplasma frutticolo autoctono dell'Etna: il melo. Atti congresso Miglioramento e valorizzazione delle produzioni frutticole Etnee 2007; 2: 7-20.

[13] De Michele A. Atti del Convegno su Germoplasma Frutticolo'. Alghero 1992.

[14] Bianco RL, Farina V, Avellone G, Filizzola F, Agozzino P. Fruit quality and volatile fraction of 'Pink Lady'apple trees in response to rootstock vigor and partial rootzone drying. J Sci Food Agric 2008; 88(8): 1325-34. [http://dx.doi.org/10.1002/jsfa.3210]

[15] Schupp JR, Fallahi E, Chun IJ. Effect of particle film on fruit sunburn, maturity and quality of 'Fuji'and 'Honey crisp' apples. Horttechnology 2002; 12(1): 87-90. [http://dx.doi.org/10.21273/HORTTECH.12.1.87]

[16] Bignami C, Scossa A, Vagnoni G. Evaluation of old Italian apple cultivars by means of sensory analysis. Acta Hortic 2003; 598: 85-90. [http://dx.doi.org/10.17660/ActaHortic.2003.598.12]

[17] Gradinariu G, Istrate M, Dascalu M, Gradinariu F. Native apple germplasm in Romania. Acta Hortic 2003; 622: 485-8. [ISHS]. [http://dx.doi.org/10.17660/ActaHortic.2003.622.53]

[18] Kellerhals M, Bertschinger L, Gessler C. Use of genetic resources in apple breeding and for sustainable fruit production. J Fruit Ornam Plant Res 2004; 12: 53-61.

[19] Farina V, Gianguzzi G, Mazzaglia A, Sortino G. Instrumental and sensory evaluation of seven apple (Malus domestica Borkh.) cultivars under organic cultivation in Sicily. In: Agronomy Research. 2017; 15: pp. (5)1878-89. [http://dx.doi.org/10.15159/AR.17.048]

[20] Fideghelli C. Valorizzare le diversità biologiche a salvaguardia delle risorse genetiche. Riv Fruttic 2007; 6: 6-7.

[21] Sansavini S, Tartarini S, Grandi M, *et al.* Nuove mele per il futuro: a confronto i programmi di selezione in corso in Italia. Riv Fruttic Ortofloric 2009; 4: 24-42.

[22] Passafiume R, Perrone A, Sortino G, *et al.* Chemical–physical characteristics, polyphenolic content and total antioxidant activity of three italian-grown pomegranate cultivars. NFS J 2019; 16: 9-14. [http://dx.doi.org/10.1016/j.nfs.2019.06.001]

[23] Guidelines for the conduct of tests Apple (Fruit Varieties) UPOV Code: MALUS_DOM (Malus domestica Borkh). Geneva, Switzerland: International Union for the Protection of New Varieties of Plants 2005.

[24] Farina V, Passafiume R, Tinebra I, Palazzolo E, Sortino G. Use of aloe vera gel-based edible coating with natural anti-browning and anti-oxidant additives to improve post-harvest quality of fresh-cut 'fuji'apple. Agronomy (Basel) 2020; 10(4): 515. [http://dx.doi.org/10.3390/agronomy10040515]

[25] Sortino G, Saletta F, Puccio S, *et al.* Extending the shelf life of white peach fruit with 1-Methylcyclopropene and aloe arborescens edible coating. Agriculture 2020; 10(5): 151. [http://dx.doi.org/10.3390/agriculture10050151]

[26] Loewus FA. Improvement in anthrone method for determination of carbohydrates. Anal Chem 1952; 24: 219. [http://dx.doi.org/10.1021/ac60061a050]

[27] AOACOfficial methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists Methods 1985a. 22.026, 22:362

[28] AOACOfficial methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists Methods 1985b. 3.140, 3: 50

[29] AOACOfficial methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists Methods 1985c.Arlington, VA, USA: Association of Official Analytical Chemists Methods 1985. 14.018, 3: 69

[30] AOACOfficial methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists Methods 1985d. 22.080, 22:369

[31] AOACOfficial methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists Methods 1985e. 22.080, 22:360

[32] Rapisarda P, Intelisano S. Sample preparation for vitamin C analysis of pigmented oral juices. Ital J Food Sci 1996; 8: 251-6.

[33] Gregori R, Farina V, Rega P, Folini L. Melo. 2015.

[34] Regolamento (CE) n 85/2004 della Commissione, del 15 gennaio 2004, che stabilisce la norma di commercializzazione applicabile alle mele.

[35] Postman J. Cydonia oblonga: The unappreciated quince. Arnoldia 2009; 67(1): 2-9.

[36] Ivascu A, Lazar V, Petrisor C. Colour variability correlated with fruit quality of different peach genotypes. Acta Hortic 2002; (592): 501-6. [http://dx.doi.org/10.17660/ActaHortic.2002.592.67]

[37] Iglesias I, Echeverría G, Soria Y. Differences in fruit colour development, anthocyanin content, fruit quality and consumer acceptability of eight 'Gala' apple strains. Sci Hortic (Amsterdam) 2008; 119: 32-40. [http://dx.doi.org/10.1016/j.scienta.2008.07.004]

[38] Angelini R. Il melo. Bayer Cropscience Srl 2008.

[39] Harker FR, Kupferman EM, Marin AB, Gunson FA, Triggs CM. Eating quality standards for apples based on consumer preferences. Postharvest Biol Technol 2008; 50(1): 70-8. [http://dx.doi.org/10.1016/j.postharvbio.2008.03.020]

[40] 2015.

[41] Gorinstein S, Zachwieja Z, Folta M, *et al.* Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. J Agric Food Chem 2001; 49(2): 952-7. [http://dx.doi.org/10.1021/jf000947k] [PMID: 11262055]

[42] Henríquez C, Almonacid S. Chi_elle, I., Valenzuela, T., Araya, M., Cabezas, L., Speisky, H. Determination of antioxidant capacity, total phenolic content and mineral composition of di_erent fruit tissue of five apple cultivars grown in Chile. Chil J Agric Res 2010; 70: 523-36. [http://dx.doi.org/10.4067/S0718-58392010000400001]

[43] Segura R, Javierre C, Lizarraga MA, Ros E. Other relevant components of nuts: phytosterols, folate and minerals. Br J Nutr 2006; 96(Suppl. 2): S36-44. [http://dx.doi.org/10.1017/BJN20061862] [PMID: 17125532]

[44] Velisek J. Davidek.Modern chromatographic analysis of vitamins. New York: Marcel Dekker 1992; p. 513.

[45] Padayatty SJ, Katz A, Wang Y, *et al.* Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr 2003; 22(1): 18-35. [http://dx.doi.org/10.1080/07315724.2003.10719272] [PMID: 12569111]

[46] Frei B. Natural antioxidants in human health and disease. San Diego, CA: Academic Press, Inc. 1994.

[47] McDermott JH. Antioxidant nutrients: current dietary recommendations and research update. J Am Pharm Assoc (Wash) 2000; 40(6): 785-99. [http://dx.doi.org/10.1016/S1086-5802(16)31126-3] [PMID: 11111359]

[48] de Lorgeril M, Salen P. Mediterranean type of diet for the prevention of coronary heart disease. A global perspective from the seven countries study to the most recent dietary trials. Int J Vitam Nutr Res 2001; 71(3): 166-72. [http://dx.doi.org/10.1024/0300-9831.71.3.166] [PMID: 11582838]

[49] Food, nutrition and the prevention of cancer A global perspective. Washington, DC: American Institute for Cancer Research 1997.