



HAL
open science

Influence of mineral fertilization (NPK) on the quality of apricot fruit (cv. Canino). The effect of the mode of nitrogen supply

Mohamed Radi, Mostafa Mahrouz, Abderrahime Jaouad, Marie Amiot

► **To cite this version:**

Mohamed Radi, Mostafa Mahrouz, Abderrahime Jaouad, Marie Amiot. Influence of mineral fertilization (NPK) on the quality of apricot fruit (cv. Canino). The effect of the mode of nitrogen supply. *Agronomie*, 2003, 23 (8), pp.737-745. 10.1051/agro:2003052 . hal-00886230

HAL Id: hal-00886230

<https://hal.science/hal-00886230v1>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Influence of mineral fertilization (NPK) on the quality of apricot fruit (cv. *Canino*). The effect of the mode of nitrogen supply

Mohamed RADI^{a,*}, Mostafa MAHROUZ^a, Abderrahime JAOUAD^a, Marie Josèphe AMIOT^b

^a Faculté des Sciences Semlalia, Département de Chimie, BP S15, Marrakech, Morocco

^b INRA, Unité Sécurité et Qualité des Produits Végétaux, Site Agroparc, 84914 Avignon Cedex 9, France

(Received 5 November 2002; accepted 14 August 2003)

Abstract – The effects of different levels of nitrogen, phosphorus and potassium (NPK) fertilizers were evaluated on biochemical markers involved in the quality of apricots (sugars, organic acids and free acidity) and technological qualities (phenolic compounds: substrates of the enzymatic browning reaction). Apricot fruits (cv. *Canino*) were picked in the Marrakech area (Morocco). An experimental design was carried out with four factors: N, P and K, each one at two levels, and two modes of N supply. The weight of fruits, refractometric index and free acidity were measured in apricots at the commercial maturity stage. The main phenolic compounds, sugars and organic acids were identified and their concentrations were determined using high performance liquid chromatography (HPLC). Apricot fruits from trees fertilized with 80 kg N ha⁻¹ showed a significantly higher content in phenolic compounds, a slightly higher sugar concentration and a lower organic acid concentration than those fertilized with 150 kg N ha⁻¹. In this latter case, the weight of fruits was higher. In contrast, fertilization with the lowest K level (60 kg ha⁻¹) resulted in lower concentrations in phenolic compounds and sugar content when compared with the fruits which received the highest K level (120 kg ha⁻¹). Our results showed that P fertilization is not the main factor in the technological qualities of apricot fruits expressed as phenolic content. The mode of N supply seemed to play a major role in the quality of apricot fruits. The second application resulted in a higher accumulation of phenolic compounds and an increase in fruit weight, while the first application tended to stimulate the biosynthesis of organic acids. In addition, our results showed great interactions between N, P and K fertilization. The N-K interaction was found to be the most significant one for all the biochemical markers studied in this work.

apricot / fertilization / experimental design / phenolic compounds / fruit quality

Résumé – Influence de la fertilisation minérale (NPK) sur la qualité de l'abricot (cv. *Canino*). Effet du mode de fractionnement d'azote.

La qualité de l'abricot, destiné notamment à subir une transformation industrielle, est fortement liée à la teneur en certains composés tels les polyphénols, principaux marqueurs de notre étude, les sucres et les acides organiques. À cet effet l'objectif du présent travail consiste à étudier l'incidence de la fertilisation minérale sur l'évolution de ces réponses dites de qualité. Cette étude a été menée dans le verger expérimental de la Société de Développement Agricole situé au sud de Marrakech (Maroc). Un plan d'expériences avec quatre facteurs : azote, phosphore et potassium, chacun avec deux niveaux, et deux modes de fractionnement d'azote (avec 1/3 d'N au mois de février et 2/3 au mois d'avril ou 2/3 d'N au mois de février et 1/3 au mois d'avril) a été mis en place. Le poids moyen du fruit, l'indice réfractométrique et l'acidité libre ont été mesurés dans les abricots récoltés à maturité commerciale. De même, les composés phénoliques, les sucres et acides organiques ont été analysés et leurs concentrations sont déterminées moyennant la chromatographie liquide haute performance (HPLC). Les résultats obtenus montrent que les fruits issus d'arbres fertilisés avec 80 kg ha⁻¹ d'azote montrent des accumulations importantes des différentes classes de composés phénoliques, des teneurs en sucres relativement élevées et des concentrations en acides organiques moindres comparativement aux fruits issus d'arbres ayant reçus une dose de 150 kg ha⁻¹. Pour cette dernière dose, le poids moyen du fruit a été trouvé plus important. La faible dose de l'engrais potassique (60 kg ha⁻¹) permet de produire des abricots avec de faibles teneurs en polyphénols et en sucres par rapport à la dose élevée (120 kg ha⁻¹). S'agissant du phosphore, l'effet de ce fertilisant est moins important sur l'élaboration de la qualité du fruit. À l'opposé, le mode de fractionnement de l'azote joue un rôle non négligeable sur les différentes réponses de qualité étudiées. Il semblerait que le deuxième apport agit plus sur l'accumulation des composés phénoliques et le poids moyen du fruit, alors que c'est le premier apport qui tend à stimuler la biosynthèse des acides organiques. Parmi toutes les interactions mises en évidence dans ce travail, l'interaction azoto-potassique apparaît jouer un rôle déterminant sur les critères de qualité du fruit. Ce résultat montre en conséquence une large interdépendance de ces deux fertilisants.

abricot / fertilisation / plan d'expériences / composés phénoliques / qualité du fruit

Communicated by Gérard Guyot (Avignon, France)

* Corresponding author: radi_mohamed@yahoo.fr

1. INTRODUCTION

Only a few studies have been devoted to the impact of different levels of mineral fertilization on the quality of apricot fruit. Different works have reported the effect of mineral fertilization on the growth and fruit yield of apricot [9, 12, 16, 17, 21, 34, 38]. Different N fertilization periods have also been tested [17, 34]. These works showed that N was weakly assimilated when supplied close to harvest. K was considered to be absorbed at high levels by apricot trees [27]. Dimitrovski and Cevtkovic [9] showed that P fertilization enhanced the growth of leaves and spurs and the yield of apricot trees. Egea et al. [11] reported that the availability of P in trees was influenced by varieties. The N-K balance was reported to greatly influence the annual yield in apricot fruits [4]. An increase in N fertilization was reported to enhance N content in apricot tree leaves and spurs [21, 38].

In general the different fertilization modes tested in apricot orchards have often consisted of evaluating separately the effect of each N, P and K fertilizer, but not their interaction effects when applied together.

In some fruit species, it has been demonstrated that mineral fertilization deficiency (particularly N) was one stress factor enhancing the content in phenolics [2, 5, 13, 24, 35, 36] and sugars [14] and, in contrast, lowering the content in organic acids [19]. In addition, fruit browning during the technological process was shown to depend on many factors, i.e. the maturity stage of the fruit, the variety and the sanitary state [20]. It has been shown that in apricot, browning was essentially related to the *ortho*-diphenolic content [37], and, more precisely, to chlorogenic acid and catechins which were found to be the best substrates for polyphenol oxidases and the limiting factors for an enzymatic browning reaction in apricot-processed products [31].

Because there is an increasing demand for apricot-processed products (juices, purées, jams and pre-cut apricots), it is necessary to obtain a high apricot fruit quality with low browning. The purpose of our study was to determine the effect of different levels of NPK on the biochemical and technological characteristics of apricot fruits, and to determine the role of each fertilizer and also the possible interactions between them. An experimental design was performed to reach this objective.

2. MATERIALS AND METHODS

2.1. Apricot tree orchard

This study was carried out in 1995 in an apricot tree orchard planted in 1980 in the Marrakech area of the Société de Développement Agricole (Morocco). The chosen cultivar was *Canino*, the most widespread cultivar in Morocco. The soil was a stony alluvial type containing 20% silt, 28% clay and 49% sand. P and K contents of the soil were evaluated [26]. At the beginning of the experiment, available P and K in the soil were, respectively, 276 $\mu\text{g g}^{-1}$ (± 21) and 470 $\mu\text{g g}^{-1}$ (± 35).

The surface area of this experimental orchard was approximately 2 ha. Twelve rows were planted north to south to optimize light interception, and trees were spaced at 4 m within the row and 6 m between rows. Guard rows were arranged to have an equal number of trees surrounding all experimental units.

The experimental design was a randomized complete block with each experimental treatment replicated three times, and each experimental unit comprising nine trees.

2.2. Application of the experimental design method

2.2.1. The model used

The objective was to measure the effect of four factors or variables (N, P, K and the mode of N supply (MNF)) on some experimental measurements (the weight of fruits, refractometric index, sugars and organic acids content, free acidity and phenolic compounds). A polynomial model was used to quantify the relationship between the value of the measurable response and a combination of experimental factors (the levels of fertilizers) presumed to affect the response.

The direct and interaction effects of factors (N, P, K and MNF) were calculated by least-squares regression using the *Nemrod* Software [25].

The phenomenon can be modeled as follows:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{14}X_1X_4 + a_{23}X_2X_3 + a_{24}X_2X_4 + a_{34}X_3X_4 + a_{123}X_1X_2X_3 + a_{124}X_1X_2X_4 + a_{134}X_1X_3X_4 + a_{234}X_2X_3X_4 + a_{1234}X_1X_2X_3X_4 + e \quad (X_i = \pm 1)$$

Y: labeled the biochemical and technological responses.

X_1, X_2, X_3 and X_4 : coded units of the variables N, P, K and the MNF.

a_0 : average of the responses.

a_1, a_2, a_3, a_4 : main effects of parameters indicating the "weight" of the variables N, P, K and the MNF, respectively.

a_{12} : two-factor interaction of N and P.

a_{123} : three-factor interaction of N, P and K.

a_{1234} : four-factor interaction of N, P, K and MNF.

e: error term (residual) supposed to be of null expected value and constant variances for the need for hypothesis testing.

2.2.2. Factor levels

Technical reports of the Société de Développement Agricole show that annual N fertilization was applied regularly with 150 kg ha^{-1} , twice in February and April alternatively with two modes of N supply (1/3, 2/3) or (2/3, 1/3). P and K were applied in December with, respectively, 90 kg ha^{-1} and 120 kg ha^{-1} doses.

The factor levels of the fertilizers and the MNF studied in this work are represented in Table I, where the two levels of each variable are identified by either a minus (–) or a plus (+) sign.

2.2.3. Choice of the experimental design

To determine simple linear models, a two-level factorial design was used as described in Box et al. [6]. With full factorial design, it is possible to determine immediately all interactions between the factors. For 4 variables, such a design will contain $2^4 = 16$ experiments. The design matrix contains 4 columns and $2^4 = 16$ rows. There is a column for each of the four

Table I. Factor levels of fertilizers (* = 1/3 of nitrogen in February and 2/3 in April, ** = 2/3 of nitrogen in February and 1/3 in April).

Factors	Symbols (variables)	Levels	
		-	+
N	X1	80 kg ha ⁻¹	150 kg ha ⁻¹
P	X2	30 kg ha ⁻¹	90 kg ha ⁻¹
K	X3	60 kg ha ⁻¹	120 kg ha ⁻¹
The mode of N supply	X4	(1/3, 2/3)*	(2/3, 1/3)**

Table II. Experimental conditions of the complete factorial matrix.

Treatment No.	N (kg ha ⁻¹)	P (kg ha ⁻¹)	K (kg ha ⁻¹)	MNF
1	150	90	120	(2/3; 1/3)
2	80	90	120	(2/3; 1/3)
3	150	30	120	(2/3; 1/3)
4	80	30	120	(2/3; 1/3)
5	150	90	60	(2/3; 1/3)
6	80	90	60	(2/3; 1/3)
7	150	30	60	(2/3; 1/3)
8	80	30	60	(2/3; 1/3)
9	150	90	120	(1/3; 2/3)
10	80	90	120	(1/3; 2/3)
11	150	30	120	(1/3; 2/3)
12	80	30	120	(1/3; 2/3)
13	150	90	60	(1/3; 2/3)
14	80	90	60	(1/3; 2/3)
15	150	30	60	(1/3; 2/3)
16	80	30	60	(1/3; 2/3)

variables and each row gives the combination of each run. The plan of experiments is shown in Table II.

For example, experiment 4 was conducted (fertilized) with the following amounts:

N: 80 kg ha⁻¹

P: 30 kg ha⁻¹

K: 120 kg ha⁻¹

MNF: (2/3, 1/3)

2.2.4. Interpretation of results

The effect of each fertilizer (expressed as coefficient response a_1 , a_2 , a_3 and a_4) and interaction effects of fertilizers (a_{ij} , a_{ijk} and a_{ijkl}) were calculated. The effect of a factor refers to the change in response when moving from the (-) to the (+) version of that factor, corresponding to low and high levels of amount, respectively. When the absolute value of the coefficient response (Tabs. IV, VI) is high, the factor has a stronger influence. A positive effect indicates that the response varies in the same sense as the factor. On the contrary, a negative effect demonstrates that the variation of this latter is in the contrary sense. The significant interactions are represented in the form of diagrams, called "interaction diagrams" (Figs. 1, 2).

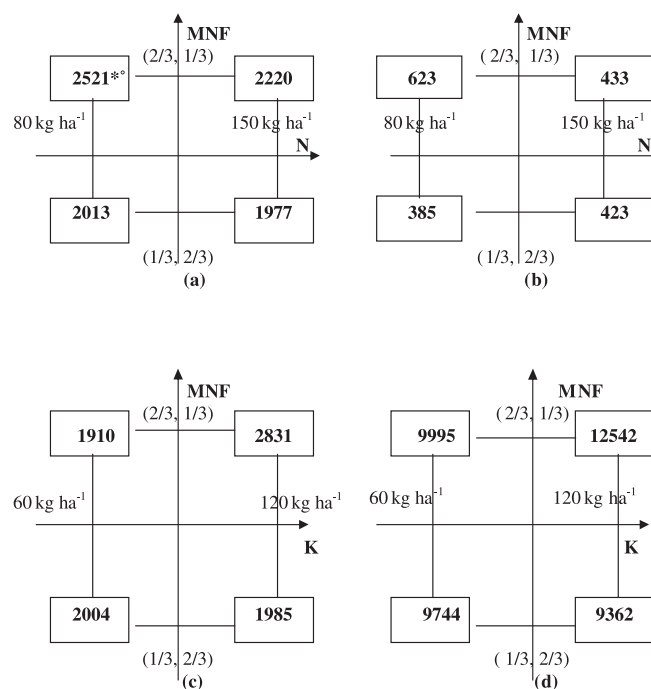


Figure 1. The incidence of N-MNF interactions on: (a): (+)-catechin concentration and (b): chlorogenic acid concentration; and of K-MNF interactions on (c): (+)-catechin concentration, and (d): FLA concentration. * Expressed in $\mu\text{g } 100 \text{ g}^{-1} \text{ FW}$. ° It is the average of the four results (concentration in (+)-catechin) obtained for the four treatments supplied with the same doses, 80N and (2/3, 1/3) MNF (treatments 2, 4, 6 and 8).

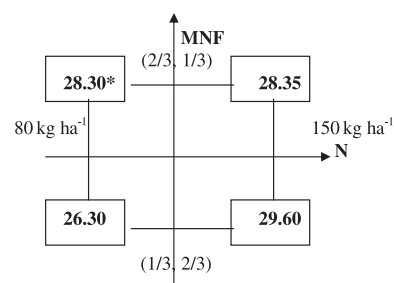


Figure 2. The incidence of two-factor interactions N-MNF on free acidity of apricot fruit. * Expressed in $\text{meq}/100 \text{ g FW}$.

2.3. Experimental methods

For each experimental unit, 300 fruits were picked on both faces of the tree (east and west) at commercial maturity and divided into two classes. Apricots from the first sample were frozen in liquid nitrogen, crushed and stored at -20°C until used. This sample was used to analyze phenolic compounds, sugars, organic acids and free acidity content. The second sample was used immediately after picking to determine the fruit weight and to measure the refractometric index.

For each analysis, measurements were done on 3 replicates.

2.3.1. Phenolic compounds

Apricot powder (10 g) was homogenized in 100 ml of cold ethanol (75%) containing sodium metabisulfite (0.5%). Three successive extractions were carried out at 4 °C for 30 min. After removal of alcohol under vacuum at 35 °C, pigments were eliminated by three successive extractions with petroleum ether (2:1, v:v). After addition of ammonium sulfate (20%) and metaphosphoric acid (2%) to the aqueous phase, phenolic compounds were extracted three times with ethyl acetate (1:1, v:v). The three organic phases were collected, combined, dried and evaporated under vacuum at 38 °C. The residue was dissolved in 1 ml methanol (1 ml). Methanolic extract was filtered through an Acrodisc filter (0.45 µm) before analysis by high performance liquid chromatography (HPLC).

Separation and determination of phenolic compounds was performed by HPLC (Varian 5500 connected to a diode array detector Waters 900) using a Chrompack C₁₈ 7 µm (200 mm × 3 mm i.d., Alltech) column. The separation and characterization conditions were similar to those previously described [31]. The concentrations of phenolics were expressed in µg equivalent chlorogenic acid for hydroxycinnamic derivatives, equivalent (+)-catechin for flavan-3-ols (catechin monomers and oligomers) and equivalent quercetin-3-rutinoside for flavonols per 100 g of fresh weight.

2.3.2. Sugars and organic acids

Apricot powder (5 g) was homogenized in 50 ml distilled water for 15 min at 4 °C using an ultra-turrax blender and centrifuged (7700 × g) for 15 min. The supernatant was treated with Sep-Pack C₁₈ and filtered on a Millipore PTFE (0.45 µm) before analysis by HPLC.

Sugars were separated by HPLC (VARIAN 9010 linked to a refractometric differential Varian RI-4) using a sugar pack column. The mobile phase (flow rate 0.5 ml·min⁻¹) consisted of water with 50 mg l⁻¹ EDTA (calcium di-sodium ethylene diamine tetra-acetate). The best separation was obtained at 85 °C and 70 atm. Sugars were assayed by external standard calculation.

Organic acids were separated using a Chrompack column. The mobile phase (flow rate 0.6 ml·min⁻¹) consisted of H₂SO₄ 0.01N. The best separation was obtained at 35 °C and 90 atm. Peaks were identified by the comparison of retention time and spectra with commercial standards. Organic acid concentrations were determined by external calibration.

2.3.3. Free acidity

Acidity was measured by titration with 0.1N NaOH and phenolphthalein as an indicator. The results were expressed as % citric acid. The supernatant obtained as described in Section 2.3.2 was used in this measurement.

2.3.4. Refractometric index

Homogenized samples were crushed with thiourea 0.5% and centrifuged (5000 × g) for 10 min. The total soluble solids was determined as °Brix expressed from each sample using a refractometer (Euromex, Holland) at 25 °C.

2.3.5. Weight of the fruits

For each experiment, 30 fruits were used to determine the mean weight of the fruits.

2.3.6. Statistical analyses

Means of data on phenolic composition, sugars and organic acids content, free acidity, the refractometric index, and the weight of the fruits were compared using Tukey's HSD [33].

3. RESULTS

3.1. Effect of NPK fertilization on phenolic content

The chromatographic separation of phenolic compounds extracted from *Canino* apricots showed that the main phenolics were identified as 5'-caffeoylquinic acid (5'CQ) (or chlorogenic acid), (+)-catechin (CAT), (-)-epicatechin (EPI) and quercetin-3-rutinoside (or rutin). Besides (+)-catechin and (-)-epicatechin, there were two oligomers (procyanidins) showing the same spectral characteristics as procyanidins B₂ and C₁ (isolated from apples and grapes [1]), comparing their retention times.

Hydroxycinnamic esters (HCE), flavan-3-ols (FLA) and flavonols (FLO) represented the three major classes of phenolic compounds for this cultivar (about 95%) (data not shown). Among the FLA, (-)-epicatechin, (+)-catechin and procyanidins B₂ and C₁ were found in high concentrations (95% of total FLA) (data not shown). Concerning the HCE, chlorogenic acid and neochlorogenic acid represented about 93% of total HCE (data not shown). Quercetin-3-rutinoside (rutin) was found to be the main flavonol with about 90% of total FLO (data not shown). Besides these major compounds, other phenolics were detected at low concentrations.

Significant differences in phenolic composition were found between the 16 treatments of NPK. HPLC analysis of phenolic compounds showed that trees fertilized with the lowest N level (80 kg ha⁻¹) produced fruits with relatively higher content in all the phenolics characterized (Tab. III). The increase in content of phenolic compounds was on average about 10% for the three main families (HCE, FLA and FLO). There were consistent differences between the two modes of N supply used in the Marrakech orchards (Société de Développement Agricole). Our results showed that when moving from the (-) to the (+) version of that factor (from 1/3N in February and 2/3N in April to 2/3N in February and 1/3N in April) the increase in the phenolic content was greater than 15% for all three families.

In contrast, fertilization with the lowest K level (60 kg ha⁻¹) resulted in lower concentration in FLA and FLO (about 5 ~ 10%), but in higher content in HCE. When moving from the (-) to the (+) version of that factor, the decrease in HCE was about 20% (Tab. III).

The analysis of the effects of NPK fertilizers and MNF on the phenolic contents (Tab. IV) showed that P fertilizer did not have a strong effect on the chlorogenic acid and FLA contents. The most influencing factors were, respectively, the MNF, N and K (the corresponding coefficients are, respectively, a₄, a₁ and a₃). This tendency was clearly shown in FLA, the major

Table III. Impact of NPK and MNF on the phenolic concentration of apricot fruits (5'CQ = 5'-caffeoylquinic acid or chlorogenic acid, CAT = (+)-catechin, EPI = (-)-epicatechin, FLA = flavan-3-ols, HCE = hydroxycinnamic esters, FLO = flavonols).

Treatment No.	5'CQ	CAT	Phenolic compounds*				Total
			EPI	HCE eq. chlorogenic	FLA eq. catechins	FLO eq. rutin	
1	272 fg**	2085 def	2197 bc	1045 efg	10092 cde	1845 c	12982 cd
2	706 b	3812 a	2757 a	1380 cd	15695 a	3743 a	20818 a
3	478 cde	3186 b	2303 b	1232 de	13469 b	1941 c	16642 b
4	434 de	2242 cd	2321 b	1159 def	10913 cd	1474 d	13546 c
5	574 c	1718 g	1873 def	1293 de	9341 de	1314 d	11948 de
6	396 def	2052 def	2175 bc	830 fgh	10059 cde	1342 d	12231 d
7	408 def	1893 fg	1819 ef	880 fgh	8772 e	2522 b	12174 d
8	958 a	1979 ef	2254 b	2301 a	11810 bc	2181 bc	16292 b
9	294 fg	2313 c	2173 bc	1331 cd	10573 cd	1666 cd	13570 c
10	215 g	2096 cde	1746 f	676 h	9699 de	1869 c	12244 d
11	521 cd	1742 g	2007 cde	1150 def	8766 e	1353 d	11269 e
12	386 def	1792 g	1728 f	908 fgh	8413 e	1830 c	11151 e
13	535 cd	2073 def	1942 def	1580 bc	9581 de	2178 bc	13339 c
14	499 cde	2040 def	2265 b	1228 de	10575 cd	2310 b	14113 c
15	342 ef	1779 g	2021 cde	816 gh	8743 e	1889 c	11448 de
16	439 de	2126 cde	2035 cde	1737 b	10079 cde	1667 cd	13483 c

*: Phenolics were expressed in µg/100 g FW.

: Mean separation within columns by Tukey's HSD, $P < 0.05$.Table IV.** The direct and interaction effects of fertilizers on the phenolic responses.

Coefficient responses	Phenolic compounds						Total
	5'CQ	CAT	EPI	HCE	FLA	FLO	
a ₀	466.00	2183.00	2101.00	1221.60	10411.30	1945.20	13578.10
a ₁	-38.06	-84.38	-59.16	-55.70	-494.10	-106.80	-656.60
a ₂	-29.69	90.63	40.00	-51.20	290.60	88.10	327.50
a ₃	-52.81	225.50	53.00	-111.50	541.30	19.90	449.60
a ₄	62.19	178.88	111.38	43.40	857.60	100.00	1001.10
a ₁₂	20.43	-142.00	-35.63	-197.60	-311.00	-175.90	-289.30
a ₁₃	16.06	7.38	75.13	-135.10	266.60	-157.10	244.60
a ₁₄	-57.19	-66.00	-105.25	-96.80	-356.30	-33.00	-486.00
a ₂₃	-11.81	77.38	24.25	49.10	271.60	227.50	548.20
a ₂₄	-11.56	-44.75	-1.88	-76.80	-262.70	-72.40	-411.90
a ₃₄	-2.94	234.88	129.13	50.50	732.10	185.60	968.30
a ₁₂₃	-87.19	-158.50	-13.63	-197.00	-643.80	-85.50	-926.30
a ₁₂₄	10.81	-222.88	-15.50	-13.10	-418.90	-165.90	-597.90
a ₁₃₄	-18.31	-52.75	-55.25	-48.10	-178.00	-60.90	-287.00
a ₂₃₄	69.56	-6.00	20.13	87.40	51.80	300.00	439.10
a ₁₂₃₄	-63.56	-144.38	-70.75	-89.50	-666.10	-164.00	-719.60

a₀: average of the response; a₁, a₂, a₃, a₄: main effects of parameters indicating the "weight" of the variables N, P, K and MNF respectively; a_{ij}: two-factor interaction; a_{ijk}: three-factor interaction; a_{ijkl}: four-factor interaction.

Table V. The incidence of NPK and MNF on the biochemical characteristics of apricot fruits.

Treatment No.	Total sugars (g/100 g FW)	Ref. Index at 25 °C	Organic acids (meq/100 g FW)	Free acidity	Weight of fruit (g/fruit)
1	6.96 c*	12.28 fgh	24.83 a	27.60 c	51.70 abcd
2	7.10 bc	12.38 efg	21.91 c	27.20 cd	39.00 f
3	7.06 bc	11.98 h	23.70 ab	28.80 b	55.00 ab
4	7.63 a	13.48 a	22.96 bc	27.80 c	41.00 e
5	6.81 cd	12.38 efg	24.44 a	29.00 b	42.80 d
6	7.46 a	12.58 def	22.94 bc	28.20 bc	49.80 abcd
7	6.35 e	12.18 gh	23.68 ab	28.00 bc	48.80 abcd
8	6.79 cd	12.68 cde	22.87 bc	30.00 ab	52.30 abcd
9	6.84 cd	12.98 bc	22.73 bc	30.00 ab	53.30 abcd
10	7.50 a	13.08 b	22.07 bc	25.80 d	47.90 bcd
11	6.53 de	12.68 cde	23.54 ab	31.40 a	55.50 ab
12	7.31 ab	11.58 i	22.84 bc	26.00 d	43.60 cd
13	6.53 de	11.58 i	22.82 bc	29.00 b	59.90 a
14	7.08 bc	12.78 bcd	21.37 c	25.80 d	48.70 bcd
15	6.63 de	12.38 efg	23.56 ab	28.00 bc	54.20 abc
16	6.95 c	12.48 defg	21.41 c	27.60 c	45.30 bcd

*: Mean separation within columns by Tukey's HSD, $P < 0.05$.

class of phenolic compounds ($a_1 = -494.1$, $a_2 = 290.6$, $a_3 = 541.3$ and $a_4 = 857.6$).

Our results showed a great interaction effect between N, K and MNF fertilization, displaying an increase or a decrease in the quality responses of the apricots. Figure 1 illustrates significant interactions between the factors (fertilizers) N-MNF and K-MNF, leading to the quantitative changes in (+)-catechin (Figs. 1a and 1c), chlorogenic acid (Fig. 1b) and FLA, which are known to be the best substrates for enzymatic browning reaction (Fig. 1d). These interactions showed a tendency to increase or decrease (+)-catechin, chlorogenic acid and FLA contents with the nature of combination between N-MNF and K-MNF (four possible combinations in each case).

Figures 1a and 1c show that the high amounts of (+)-catechin were obtained, respectively, with the combinations of fertilizers (N: 80 kg ha⁻¹; MNF: (2/3, 1/3)) and (K: 120 kg ha⁻¹; MNF: (2/3, 1/3)). The scores obtained in these cases were increased by about 28% and 48%, respectively, as compared with the combinations (N: 150 kg ha⁻¹; MNF: (1/3, 2/3)) and (K: 60 kg ha⁻¹; MNF: (2/3, 1/3)).

Figure 1b indicates that when 150 kg ha⁻¹ of N fertilizer was applied, the MNF had no effect on chlorogenic acid concentration. However, when 80 kg ha⁻¹ was applied, chlorogenic acid content was increased by more than 60% when moving from the (-) to the (+) version of the MNF.

Figure 1d shows that when 60 kg ha⁻¹ of K was applied, the MNF had only a weak effect on FLA concentration. But, this effect was stronger when K treatment was applied at 120 kg ha⁻¹.

Consequently, phenolic contents obtained by application of a single fertilizer were markedly different from those obtained when fertilizers were applied in combination.

3.2. Sugars and refractometric index

Total sugars ranged between 6.35 and 7.63 g/100 g fresh weight for the different treatments applied (Tab. V). The chromatographic separation of sugars extracted from the apricot fruits showed that the major sugars were saccharose, glucose, fructose and sorbitol. Saccharose concentration was greater than 80% of total sugars, glucose concentration approximately 10%, fructose concentration greater than 5% and sorbitol concentration lower than 3% (data not shown).

Soluble solids expressed as a refractometric index ranged between 11.58 and 13.48 at 25 °C.

The data reported in Table V indicated that there were no significant differences between samples from the sixteen experiments.

N fertilization showed the strongest negative effect on sugar concentration ($a_1 = -0.26$ for sugars and -0.16 for the refractometric index) (Tab. VI). The lowest sugar amounts were obtained with 150 kg ha⁻¹ N treatments. All the fruits from the 80 kg ha⁻¹ N treatment had a minimum 8% higher average sugar concentration than those from the other treatments (Tab. V).

The second influencing factor was K, which displayed a positive effect on the accumulation of sugars ($a_3 = 0.14$ for sugars and 0.09 for the refractometric index, Tab. VI). An increase in sugars and in the refractometric index from the 60 kg ha⁻¹ K level to the 120 kg ha⁻¹ K level was observed. Thus, the highest score for sugars content was 7.63 obtained for experiment 4 conducted with 80 kg ha⁻¹ N and 60 kg ha⁻¹ K (Tab. V).

In contrast, for P fertilization and MNF, no significant differences were detected, respectively, between 30 kg ha⁻¹ P

Table VI. The direct and interaction effects of fertilizers on the biochemical responses of apricot fruits.

Coefficient responses	Total sugars	Ref. Index	Organic acids	Free acidity	Weight of the fruits
a ₀	6.97	12.47	22.98	28.14	49.30
a ₁	-0.26	-0.16	0.68	0.84	3.35
a ₂	0.06	0.04	-0.09	0.19	-0.16
a ₃	0.14	0.09	0.09	-0.06	-0.92
a ₄	0.05	0.03	0.44	-0.31	-1.75
a ₁₂	0.01	-0.04	0.13	0.23	-0.56
a ₁₃	-0.01	0.09	-0.06	0.53	2.15
a ₁₄	0.03	-0.12	0.06	-0.81	-1.33
a ₂₃	-0.08	0.09	-0.10	0.11	-0.24
a ₂₄	0.00	-0.12	0.20	-0.01	-1.56
a ₃₄	0.02	-0.05	-0.16	-0.41	0.05
a ₁₂₃	0.06	-0.06	0.13	0.46	0.41
a ₁₂₄	0.02	-0.25	0.22	-0.04	0.04
a ₁₃₄	0.06	0.20	0.22	0.21	-2.50
a ₂₃₄	-0.14	0.20	0.00	0.01	-0.64
a ₁₂₃₄	0.02	0.07	0.05	0.04	0.69

a₀: average of the response; a₁, a₂, a₃, a₄: main effects of parameters indicating the "weight" of the variables respectively; N, P, K and the MNF; a_{ij}: two-factor interaction; a_{ijk}: three-factor interaction; a_{ijkl}: four-factor interaction.

and 90 kg ha⁻¹ P and between (1/3, 2/3)MNF and (2/3, 1/3) MNF levels. The effect coefficients of these two factors (respectively, a₂ = 0.06 and a₄ = 0.05 for sugars and a₂ = 0.04 and a₄ = 0.03 for the refractometric index, Tab. VI) were low.

Besides these two direct effects of P and MNF, the interactions between fertilizers (in particular the two-factor interactions) were not significant in regard to the refractometric index and sugars concentration.

3.3. Organic acids and free acidity

The chromatographic separation of organic acids extracted from the apricot fruits showed that the main acids detected were citric and malic acids. Succinic, quinic and shikimic acids were also detected. Citric acid reached more than 60%, malic acid about 30% and the three other acids represented about 8% of the total acids (data not shown).

Total organic acids were in the range of 21.37–24.83 meq/100 g FW for the different treatments applied. The free acidity expressed as citric acid ranged between 25.8 and 31.4 meq/100 g FW (Tab. V).

It can be easily seen that all the fruits from the 150N treatment displayed significantly higher scores in terms of total organic acids and free acidity (Tab. V). For example, the decrease in free acidity was more than 18% between trial 11 (150N) and trial 12 (80N).

In contrast, there was not a great influence in regard to organic acids and free acidity for P and K fertilization, respectively, between 30 kg ha⁻¹ P and 90 kg ha⁻¹ P and between

60 kg ha⁻¹ K and 120 kg ha⁻¹ K levels (respectively, a₂ = -0.09 and a₃ = 0.09 for organic acids and a₂ = 0.19 and a₃ = -0.06 for free acidity, Tab. VI).

The direct effects of the factors NPK and the interactions between them on organic acids and free acidity responses are reported in Table VI. The results indicated that the effect of N (a₁ = 0.68 and 0.84) and the mode of N supply (a₄ = 0.44 and -0.31) and the interactions between them concerning free acidity response (a₁₄ = -0.81, Tab. VI) are the main effects. The other direct effects or interaction effects were less influential.

Figure 2 shows that for the interaction N-MNF, the biggest score of free acidity was obtained with the combination 150N and (1/3, 2/3) MNF.

3.4. Average fruit weight

The average fruit weight was in the range of 39–59 g/fruit for the sixteen experiments. The lowest average fruit weights were obtained with the 80N treatment (Tab. V). Our results showed that fruits from the 150 kg ha⁻¹ N treatment were about 18% heavier than fruits from the 80 kg ha⁻¹ N treatment when the MNF was (1/3, 2/3), and 8% when the MNF was (2/3, 1/3). For example, the decrease in average fruit weight was about 25% between trial 1 (150N) and trial 2 (80N). Table V shows that the mode of N supply seemed to play a major role in fruit weight. For 150 kg ha⁻¹ N, fruits from the (1/3, 2/3) treatment were heavier than fruits from the (2/3, 1/3) treatment by about 13%, whereas no significant fruit weight differences were detected according to P and K treatments (respectively, a₂ = -0.16 and a₃ = -0.92) (Tab. VI).

Among the two-factor interactions illustrated for this response, $a_{13} = 2.15$, $a_{14} = -1.33$ and $a_{24} = -1.56$ appeared more important than the others (Tab. VI). This data indicated that all fertilizers (NPK and MNF) presented direct or indirect effects on apricot fruit weight.

4. DISCUSSION

The quantitative effects of N, P and K on phenolic contents were determined and the interactions between all the fertilizers were quantified. Thus, the phenolic content of apricot fruits could be substantially modified by cultural practices. Higher supplies in N have already been reported to decrease the phenolic content of different fruit species [2, 18, 20, 35]. Our results confirmed this tendency in apricot fruits, and gave some details about NPK fertilization incidence on the content of the three major classes of phenolics in apricot and the two main phenolics involved in browning.

In addition, our experiments gave new data on the mode of N supply. This factor appeared to have a high influence on the changes in phenolic concentrations. Phenolic content increased when the second supply of N (in April) was lower than the first. This result underlines that the phenolic biosynthesis was accelerated just before the maturation stage and under N deficiency conditions. Previous works indicated an accumulation of phenolic compounds under N stress. Excess carbon skeletons appeared to be produced when phenylalanine was degraded to supply ammonia [22, 23]; leaf proteins are hydrolyzed under N stress to supply amino acids for processes related to root growth which may increase N availability [5].

Furthermore, a slight level of K fertilization (60 K) contributed to enhancing the HCE content of apricots, when compared with the highest level (120 K). This is the first time that such data have been reported. Our results have to be confirmed with different K amounts. In contrast, fertilization with 120 kg ha⁻¹ increased FLA, and to a lesser extent, FLO concentration. The effect of K on phenolic compound accumulation in plants has not previously been reported. Only some general observations were described in some fruit species as summarized by Macheix et al. [20]. The effect of this fertilizer was evaluated in this work by focusing on its interactions with N fertilization. In parallel, our results show that P fertilization did not considerably modify the phenolic content. Similar results were obtained with 30 kg ha⁻¹ P and 90 kg ha⁻¹ P levels. This factor presented the weakest coefficient responses comparatively to the other factors. Possible reasons for this result could be advanced: (i) firstly, the factor levels of P choice in these experiments (used in the Société de Développement Agricole orchard) were not very significant to influence responses; (ii) secondly, P was not absorbed by the apricot trees in significant quantities, unlike N and K. Nevertheless, this result was not surprising. P fertilization has been reported to act essentially on vegetative growth [9]. The same result was also observed in some fruit species [29, 30].

In addition, it was not previously demonstrated that the interaction between fertilizers could have a significant impact on phenolic content. With our experimental design such interactions were evaluated. N-MNF and K-MNF significant interactions were shown, leading to a modulation of the content in

catechins, chlorogenic acid and FLA. Specifically, 120 kg ha⁻¹ K increased FLA content at (2/3, 1/3) MNF and reduced it at (1/3, 2/3) MNF (Fig. 1d). Similarly, 80 kg ha⁻¹ N enhanced chlorogenic acid concentration at (2/3, 1/3) MNF and decreased it at (1/3, 2/3) MNF (Fig. 1d).

Concerning the incidence of NPK and MNF on the biochemical characteristics of apricots, sugars decreased when N supply increased. The opposite effect was observed for acidity, as described in grapes [32] or in peaches [19]. In the same way, N at 150N tended to increase average fruit weight. This tendency was more pronounced when the mode of N supply was (1/3, 2/3). All the fertilizer combinations studied in this work produced apricots with acceptable taste according to data published by Audergon et al. [3]. Moreover, a remarkable result was obtained in regard to the effect of the MNF on the organic acids content in apricots. The coefficient response of that factor ($a_4 = 0.44$; positive effect) showed that the (2/3, 1/3) MNF level produced more organic acids than the (1/3, 2/3) MNF level. Organic acids were accumulated in the growth stage (first application). These data were also reported in peach fruits [7, 8, 19], indicating that the organic acids accumulation was accelerated in the early stages of the fruit.

In our experiment, K fertilization seemed to influence positively the sugar content, as previously described by Mengel and Haeder [27] and Ho and Baker [15]. However, moderate average fruit weight variation and no significant acidity change were revealed with variations in levels of P and K fertilization. These results are in good agreement with previously reported data for apple [28, 40], but differ from results published on other fruits such as peach [10] and pear [39], where K fertilization increased acidity. In the present study, the high level of K in the soil and an average supply of P at the beginning of the experiment may explain this lack of response.

5. CONCLUSION

N fertilization appeared to have stronger effects on many quality markers than P and K fertilization. Among the different experiments, 150 kg ha⁻¹ N with the (1/3, 2/3) MNF appeared particularly adequate to favor the processed apricot quality, since fruits with a lower content in phenolic oxidizable substrates could be less susceptible to enzymatic browning. In addition, the highest average fruit weights were obtained with the 150N treatment. In contrast, trees fertilized with 80 kg ha⁻¹ N, which is the most environmentally-friendly, produced commercial fresh apricots relatively rich in phenolic compounds known to have health benefits because of their antioxidative properties. Further experiments with intermediate N amounts would be necessary to confirm the optimal N fertilization.

Concerning P and K, these two fertilizers (with the levels used in this work) did not markedly modify the phenolic content of apricot fruits. However, further studies are needed to clearly specify the incidence of K using different levels of this fertilizer.

N and K interacted to affect the quality of apricot. Thus a N-MNF and a N-K fertilization balance appears necessary to improve the technological quality of apricot fruits.

The organization of the present trials according to the experimental design would seem to facilitate the simultaneous study of the effect of the fertilizers NPK on the commercial quality of Moroccan apricots (cv. *Canino*). This method can be extended to a greater number of agronomic factors in order to show the effect of each of them as well as the interactions between them.

Acknowledgements: This work was funded by the "Ambassade de France" in Morocco. We thank Max Tacchini and Maryse Reich for technical assistance and the Société de Développement Agricole staff for their help and material support during this work.

REFERENCES

- [1] Amiot M.J., Tacchini M., Aubert S., Nicolas J., Phenolic composition and browning susceptibility of various apple at maturity, *J. Food Sci.* 57 (1992) 958–962.
- [2] Andrew G.H., Beech F.W., The phenolic of ciders: effect of cultural conditions, *J. Sci. Food Agric.* 29 (1978) 493–496.
- [3] Audergon J.M., Souty M., Breuils L., Amélioration génétique pour l'obtention d'abricots de qualité, in: INRA-CTIFL (Ed.), *La maîtrise de la qualité des fruits frais*, 9^e Colloq. sur les Recherches fruitières, Avignon 4–5–6 décembre, 1990, pp. 217–228.
- [4] Balo E., Panczel M., Prileszky G.Y., Rational fertilization of apricot trees on the basis of leaf diagnosis, *Acta Hortic.* 85a (1981) 491–497.
- [5] Bongue-Bartelsman M., O'Neill S.D., Tong Y., Yoder J.L., Characterization of the dihydroflavonol 4-reductase gene in tomato, *Gene*. 138 (1994) 153–157.
- [6] Box G.E.P., Hunter W.G., Hunter S., *Design For Experiments*, J. Wiley (Ed.), 1976.
- [7] Chapman G.W., Horvat R.J., Changes in nonvolatile acids, sugars, pectin, and sugar composition of pectin during peach (cv. *Monroe*) maturation, *J. Agric. Food Chem.* 38 (1990) 383–387.
- [8] Chapman G.W., Horvat R.J., Forbus W.R., Physical and chemical changes during the maturation of peaches (cv. *Magestic*), *J. Agric. Food Sci.* 39 (1991) 867–870.
- [9] Dimitrovski T., Cevetkovic D., The effect of NPK on the growth, yield and quality of the apricot fruit, *Acta Hortic.* 85a (1981) 481–489.
- [10] Du Preez M., Effect of fertilisation on fruit quality, *Decid. Fruit Grow.* (1985) 138–140.
- [11] Egea L., Leon A., Carpena O., Nutrition minérale chez certains *Prunus* L. Influence de l'espèce, *Fruits* 27 (1972) 287–291.
- [12] Faust M., Modern concept in fruit nutrition, in: Atkinson D., Jackson J.E., Sharples R.O. (Eds.), *Mineral nutrition of fruit trees*, Butterworths, London, 1980, pp. 11–15.
- [13] Francis F.G., Atwood W.M., The effect of fertilizers treatments on the pigment content of cranberries, *Am. Soc. Hortic. Sci.* 77 (1960) 351–358.
- [14] Habib R., Triboui E., Génard M., Le Bail M., La nutrition azotée des cultures et la qualité des produits, in: INRA (Ed.), *Maîtrise de l'azote dans les agrosystèmes*, les colloques, n° 83, Paris, 1997, pp. 141–159.
- [15] Ho L.C., Baker D.A., Regulation of loading and unloading in long distance transport systems, *Physiol. Plant.* 56 (1982) 225–230.
- [16] Kotze W.A.G., Joubert M., Effect of different organic materials for soil improvement, mulching and fertilization on the performance of apricot trees, *J. South. Afr. Soc. Hortic. Sci.* 2 (1992) 31–35.
- [17] Kotze W.A.G., Villiers J., Uptake of ¹⁵N labelled ammonium and nitrate by apple, apricot and nectarine trees, *J. South. Afr. Soc. Hortic. Sci.* 1 (1991) 89–91.
- [18] Koumba-Koumba D., Macheix J.J., Biosynthesis of hydroxycinnamic derivatives in apple fruit cell suspension culture, *Physiol. Vég.* 20 (1980) 137–142.
- [19] Lobit P., Étude et modélisation de l'acidité des pêches (*Prunus Persica* L. Batsch, cv. *Fidelia*) : Application à l'étude des effets de la nutrition azotée, Thèse de Doctorat, ENSAM, Montpellier, 1999, 231 p.
- [20] Macheix J.J., Fleuriot A., Billot J., *Fruit Phenolics*, CRC Press, Inc., Boca Raton, Florida, 1990.
- [21] Margarian A.A., Maroutian S.A., Michaelian V.M., Nitrogen metabolism in apricot as a function of mineral nutrition, *Acta Hortic.* 192 (1986) 35–40.
- [22] Margna U., Control at the level of substrate supply – an alternative in the regulation of phenylpropanoid accumulation in plant cells, *Phytochemistry* 16 (1977) 419–426.
- [23] Margna U., Margna E., Vainjarv T., Influence of nitrogen nutrition on the utilization of L-phenylalanine for building flavonoids in buckwheat seeding tissues, *J. Plant Physiol.* 134 (1989) 697–702.
- [24] Marigo G., Boudet A.M., Relations polyphénols-croissance : mise en évidence d'un effet inhibiteur des composés phénoliques sur le transport polarisé de l'auxine, *Physiol. Plant.* 41 (1977) 197–202.
- [25] Mathieu D., Phan Tan Luu R., Logiciel Nemrod, Université d'Aix-Marseille III, 1980.
- [26] Mehlich A., Mehlich III soil test extractant: a modification of Mehlich II extractant, *Commun. Soil Sci. Plant Anal.* 15 (1984) 1409–1416.
- [27] Mengel K., Haeder H.E., Effect of potassium supply on the rate of phloem sap exudation and the composition of phloem sap, *Plant Physiol.* 59 (1977) 282–284.
- [28] Noe N., Eccher T., Porro D., Stainer R., Quality of Golden Delicious apples as affected by season and by nitrogen and potassium mineral nutrition, *Acta Hortic.* 448 (1997) 487–497.
- [29] Plenet D., Etchebest S., Mollier A., Pellerin S., Growth analysis of maize field crops phosphorus deficiency: I. Leaf growth, *Plant and Soil* 223 (2000) 117–130.
- [30] Preters J., Aandacht voor fosfaat werp harde vruchten af, *Groenten en Fruit* 27 (1991) 8–9.
- [31] Radi M., Mahrouz M., Jaouad A., Tacchini M., Hugues M., Amiot M.J., Phenolic composition, browning susceptibility and carotenoid content on several apricot cultivars at maturity, *HortSciences* 32 (1997) 1087–1091.
- [32] Ruhl E.H., Effect of potassium and nitrogen supply on the distribution of minerals and organic acids and the composition of grape juice of Sultana vines, *Aust. J. Exp. Agric.* 29 (1989) 133–137.
- [33] SAS Institute, SAS user's guide: statistics. Version 5. SAS Inst., Cary, NC, 1985.
- [34] Sud G., Bhutani V.P., Effects of autumn sprays of urea on the nutrient status of apricot, *Hortic. J.* 7 (1994) 83–88.
- [35] Suniaga Quijada J.S., Nutrition azotée de la tomate du type déterminé issue de semis. Analyse de la croissance et de développement au stade jeune, Thèse de Doctorat, Université Rennes I, Rennes, 1991, 173 p.
- [36] Tan S.C., Phenylalanine ammonia-lyase and the phenylalanine ammonia-lyase inactivating system: effects of light, temperature and mineral deficiencies, *Aust. J. Plant Physiol.* 7 (1980) 159–167.
- [37] Vamos-Vigyazo L., Nadudvari-Markus V., Gajzago I., Polyphenol oxidase and peroxidase activities polyphenol complex of apricot cultivars, 19th annual meeting, Budapest, Hungary, June 25–29, Proc. Hung. Annu. Meet. Biochem. 19 (1979) 221–222.
- [38] Watanabe T., Tanabe K., Fukui H., Nakamura M., Relation between fruit yield and amino acid and carbohydrate contents of leaves and spurs of Japanese apricot tree, *J. Jap. Soc. Hortic. Sci.* 59 (1990) 341–347.
- [39] Wooldridge J., Determination of the fertilizer requirements of Pear trees from tree performance, leaf analysis and fruit quality data, *Decid. Fruit Grow.* 44 (1994) 28–31.
- [40] Wooldridge J., Olivier C.M., Effect of phosphorus on starking apple, *Decid. Fruit Grow.* 45 (1995) 31–34.