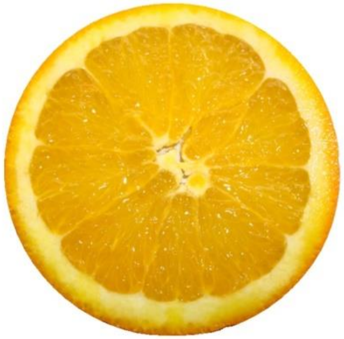

Increasing the quality of juices

Decreasing the production cost and analysis costs

PHYSICOCHEMICAL CHARACTERISTICS AND CRITERIA FOR EVALUATION OF IDENTITY OF THE SPANISH LEMON JUICE.

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ABSTRACT

The purpose of this research was to analyze the juice lemon, obtained in the industry and in the plant pilot. The results of this study support that physicochemical characteristics and the antioxidant capacity of lemon juice differ depending on the geographical origins. The juice of Spain contains 344,85-513,52mg/Kg of ascorbic acid. The citric acid/isocitric acid ratio of Spain juices has values in the higher range (120-350). The juice of Spain contains 252,21-399,47mg/L of hesperidin. The minimum level of interval of serine, aspartic acid, glutamic acid, alanine, glycine, and threonine is reduced on the comparison with the minimum AIJN*. Intervals of valine and leucine are increased on the

comparison with the AIJN. Range values of mineral concentrations of Spain samples were higher and larger than its values of “AIJN”.

INTRODUCTION

Citrus juices have been the subject of many studies (Mouly et al., 1994). Lemons (Citrus Limon Burn; Rutaceae) are cultivated in many countries all over the world, in regions with temperate summers and mild winters, particularly in Mediterranean countries, southern California, and Argentina. The phytochemical composition and bioactivity of lemon juices depend on the geographical origins. Values of the phytochemical composition suggested that the geographical origins have the most effect on differentiation of lemon juices. The presence of bioactivity components increases the antioxidant capacity of the juice.

During the last years, research in the field of nutrition and cancer causation has led to exciting, significant progress in providing an understanding of specific risk factors and identifying potential preventive agents in the diet. D-Limonene, which comprises >90% of citrus peel oil, is of significant interest because it has the capacity to inhibit the carcinogenesis processes via a variety of mechanisms. In preclinical studies in patients with advanced cancer the maximum tolerable oral dose applied for several months was 13,8 g limonene per day. In some cases, tumor growth was inhibited (Schmandke, H., 2003). D-

Limonene, it is found naturally in orange juice at an average concentration of 100 mg/liter (H-H. Sherry, et al., 2002).

In the lemon juice of Italy content total of 35 components (10 monoterpene hydrocarbons, four sesquiterpene hydrocarbons, seven monoterpene alcohols, seven aldehydes, five esters, and two miscellaneous) were identified in lemon juices. Monoterpene hydrocarbons (limonene, α-thujene, α-pinene, camphene, β-pinene, β-myrcene, α- terpinene, β-ocimene, γ-terpinene, and terpinolene) constitute the main volatile group, representing 70-90% of total volatiles in all four juices. Limonene is by far the most abundant monoterpene in all juices (54,1-68,8% of total), followed by γ-terpinene (6,8-11,4%), β-pinene (2,6-6,5%), terpinolene (1-1,7%), and β-myrcene (1-1,5%). Sesquiterpene hydrocarbons (β-caryophyllene, α-bergamotene, α-zingiberene, and β-bisabolene) are the second most important with β-bisabolene representing 0,3-3,4% and α-bergamotene representing 0,2-1,9% of the total. Oxygenated compounds are mainly aldehydes (nonanal, decanal, 9-methyldecanal, undecanal, dodecanal, neral, and geranial), monoterpene alcohols (linalool, fenchol, borneol, 4-terpineol, α-terpineol, nerol, and geraniol), and esters (octyl acetate, nonyl acetate, citronellyl acetate, neryl acetate, and geranyl acetate) (4,20%-24,28%). The monoterpene alcohols show a similar trend of 19,29; 8,17; 10,77 and 3,00% of the volatile compounds in the four juices (Gianna et al., 2006).

The following monoterpene hydrocarbons were found in the North East Tunisia juice analyzed: limonene (78,84%), ocimene (3,85%), p-cymene (1,75%), α- terpinene (0,46%), α-pinene (0,27%) and β-pinene (0,02%). Volatile compounds have been identified in the North East Tunisia juice analyzed such as methanol, isopropanol, butyl acetate, 3-heptanone, nonanol, linalool, α-terpineol, valencene, β-ionone and terpinen-4-ol. The freshly harvested lemon juice contains 75,93g/L of total sugars, 53,30 mg /mL of citric acid and 3,50 mg/mL of malic acid. In addition, the quantification of fatty acids in citrus juices points out the abundance of 4 fatty acids (palmitic, oleic, linoleic and linolenic) constituting 71.24 of the total acids in lemon juice (C16:0: 14.20%, C18:1: 21.04%, C18:2: 26.22%, C18:3: 9.77%) (Saïdani Moufida.2003).

An analytical differentiation of lemon juices, based on the differing concentrations of certain minor components, including flavonoids, plays an important role in determining chemotaxonomic markers to ascertain the authenticity of commercial products.

Lemon juice (California) contains such biofunctional components as flavonoids, carotenoids, and ascorbic acid (AA*). Eriocitrin (eriodictyol 7-O-β-rutinoside - 216 μg/g of juice) and hesperidin (hesperetin 7-O- β-rutinoside - 197 μg/g of juice) among the flavanone glycosides are contained abundantly in lemon juice (Yoshiaki, M., and et al. 2007).

It has been previously demonstrated that hesperidin and diosmin show therapeutic effects on microcirculation in chronic venous insufficiency (Struckmann, J. R., 1994). Eriocitrin has shown a suppressive effect against oxidative stresses caused by exercise and diabetes (Miyake, Y., et al. 1998; Minato, K., et al. 2003). Further more, combined treatments of diosmin and hesperidin showed beneficial properties against oxidative stress and decreased the internal hemorrhoids of pregnancy (Buckshee, K, et al. 1997).

Phenolic compounds, such as 1-feruloyl-β-D-glucopyranoside (17,10 μg/g of juice); 1-sinapoyl- β-D-glucopyranoside (10,30 μg/g of juice); 6,8-C- β-diglucosylapigenin (11,50 μg/g of juice); 6,8-C- β-diglucosyldiosmetin (57,90 μg/g of juice) among certain minor components are contained in California lemon juice (Yoshiaki, M., et al. 2007). These four compounds exhibited lower radical scavenging activity than eriocitrin, a potent antioxidant in lemon fruit. 6,8-Di-C-glucopyranosylapigenin and 6,8-di-C-glucopyranosyldiosmetin were detected in a variety of Southern Italian Citrus juices (orange, lemon, bergamot, citron, mandarin, clementine). From the (Corrado Caristi et al., 2006) results it appears that while 6,8-di-C-glucopyranosyldiosmetin may be considered a characteristic component of lemon juices. In fact, the concentration of 6,8-di-C-glucopyranosyldiosmetin (36–60 mg/L) in lemon juice was comparable with the diosmin content (32–67 mg/L) (Caristi et al., 2003). Extensive in vivo and in vitro experiments of these showed beneficial health activities as protective agents against cancer (Koyuncu, H.; et al. 1999) and cardiovascular (Borradaile, N.M.; et al. 1999), inflammatory (Galati, E. M.; et al. 1994).

Juice from lemon of China is investigated mainly on quality parameters (ascorbic acid -233,44 mg/L, total phenolics -751,82 mg/L, hesperidin-237,96 mg/L, total phenolic acids (caffeic, p-coumaric, ferulic, sinapic, protocatechuic, p-hydroxybenzoic, vanillic) - 58,50 mg/L of juice) (Guihua X., et al.2008)

Antioxidant capacity of citrus juice of China was evaluated by FRAP (AEAC) and DPPH assay (1%). Lemon had the lowest value of 307,43 AEAC mg/L. Contribution of AA to total antioxidant capacity was calculated, and it was found that AA contribution to total antioxidant capacity of citrus juices was more than 50%. The results were in agreement with previous reports (Arena et al., 2001; Caro et al., 2004; Gardner et al., 2000), which suggested AA, not phenolic compounds, was the major contributor of total antioxidant capacity of citrus juices. However, some studies suggested phenolic compounds dominated total antioxidant capacity of citrus fruits (Wang et al., 1996).

The purpose of this research was to analyze the lemon juice, obtained in the industry and in the pilot plant. The present study was compared lemon “Fino” y lemon “Verna”, established in southeastern Spain, and was aimed at characterizing the composition of their juices and finding possible relationships between phytochemical composition of lemon juices and the geographical origins.

MATERIALS AND METHODS

Lemon juices were obtained from fruits in the period December 2002 to July 2005 directly from commercial processing plants and at the experimental farm of the Universidad Miguel Hernandez, Spain.

Titrable acidity was calculated as percentage of citric acid by titrating 10mL of the lemon juice with a solution of NaOH (0.1N) till pH=8.1.

The pH was measured by a pH meter.

The level of sugars was measured as °Brix by a digital refractometer. To determine the content of sugars and organic acids in juice, samples (1mL) were centrifuged for 20 minutes at13000rpm and filtered through a 0.45μm filter (Millipore).

The composition of sugars and acids was detected with an HPLC Refractive index (RI) 1530 detector. The different sugars and organic acids were identified by comparison of their retention times with those of pure standards. The concentrations of these compounds were calculated from standard curves of the respective sugars and organic acids. Extraction of volatiles was accomplished with a 50:50 mixture of pentane and diethyl ether. Four milliliters of pentane/ether solution and an internal standard, 6L of 100 ppm propylbenzene, were added to 4 mL of juice and mixed using a Mixxor-like apparatus. The emulsion was broken us ingarefrigerated centrifuge for 10min (15000g). Approximately 3 mL of the pentane/ether layer was collected and concentrated to30L for analysis. The concentrated extract was immediately injected into the Gas Chromatographic (GC) Analysis.

The individual volatile constituents were separated with an HP-5890GC utilizing a flame ionization detector (FID) using a 30 m 0.25 mm i.d. 0.5m film thickness low-bleed DB-5 column. The oven temperature was programmed from 35 to 275 °C at 6 °C/min with helium at a flow rate of 1.55 mL/min. Injector temperature was maintained at 250 °C and detector temperature at 320 °C. Nitrogen gas was maintained at 19 mL/min, while air and hydrogen were maintained at 296 and 35mL/min, respectively.

RESULTS AND DISCUSSIONS

Fresh juices of Spain cultivars lemons, named of lemon “Fino” and lemon “Verna”, have been analyzed by methods of AIJN. The composition of lemon juice depends from cultivar type, environmental factors and postharvest factors, storage and processing factors. Physiologic active substances in lemon juices were examined during December -July period of the 2002-2005 seasons. Special attention was paid to the level of monoterpene, free amino acids, flavonoides, organic acid, sugars, and mineral composition in industry juices and pilot plant juices. A total of 47 compounds have been identified. Identified compounds and their relative amounts in mg/Kg, mg/L, and (%) are given in Tables 1, 2, 3, 4, 5 and in Fig. 1, 2, 3, 4, 5.

Glucose was the most dominant sugar in the lemon juice. Results of our studies show that the average concentration of glucose content ranged from 7,63 to 8,47(g/L) in the juice industry and from 7,52-8,16(g/L) in the pilot plant juice. The maximum concentration of glucose of AIJN is 12,00 and the minimum is 3,00 (g/L) (Table 1, 2). Followed by fructose was. The average concentration of fructose content ranged from 7,27-8,00 (g/L) in the industry juice and from 6,94-7,48(g/L) in the pilot plant juice. The maximum concentration of fructose of AIJN is 11,00 and the minimum is 3,00 (g/L). Followed by sucrose was. The level of average concentrations was 3,05- 4,00 (the industry juice) and 3,60-5,18 (the pilot plant juice). The level of sucrose concentration of AIJN was 7,00 and 0 (g/L).

Data of the organic acids detected in the industry juice and in the pilot plant juice are summarized in the Table 1, 2. The citric acid was the principal organic acid. The average concentration of citric acid of the industry juice achieved 48,26-54,06g/Kg and the average concentration of the pilot plant juice achieved 51,05-54,61g/Kg. The maximum concentration of citric acid of AIJN is achieved 63,00g/Kg and the minimum is achieved 45,00g/Kg. Followed by malic acid was. The level of average concentrations was 1,22-2,68g/Kg (of the industry juice) and 1,42-2,53g/Kg (of the pilot plant juice). The maximum concentration of malic acid of AIJN is 7,50g/Kg and the minimum is 1,00g/Kg. The lower content of isocitric acid was found through samplings for the juice of industrial and juice of pilot plant (208-308mg/Kg and 226-406mg/Kg, respectively). The maximum concentration of AIJN is 500mg/Kg and the minimum is 230mg/Kg (Table 1).

According to Reference Guideline for "Lemon Juice" the concentration of citric acid correlates with the isocitric acid concentration to a certain extent. The ratio can be used to detect an acidification with citric acid. The results of our studies (2002-2005 years) show that the citric acid/isocitric acid ratio of juices from Spain has values in the higher range (120-350) (Fig. 1). The ratio of citric acid/isocitric acid of juices from South America, California and Israel are below that 200 (AIJN) (Table 1).

Industrially processed juices showed almost no deviation from the range limits of Formol number (13-26 mL 0.1M NaOH/100) (AJIN). However, the range of lemon juice from Spain has the value attained 12-28 ml 0.1 M NaOH/100 (Fig. 2).

It's well known that vitamin C and carotenoids are abundant in some citrus fruits (Dhuique-Mayer, et al. 2005), thus they are very beneficial to human health. Values presented of vitamin C components (AA, dehydroascorbic acid-DHAA) they were in agreement with values Lee, S.K (Lee, S.K et al., 2000), where AA was the predominant component of total vitamin C in lemon juice. Changes of AA the sampling periods (from December 2002 to July 2005) are shown in Tables 1, 2. The results of our studies show that, the average AA content ranged from 344,85mg/Kg to 513,52mg/Kg for Spanish lemon industry juice and the average AA content ranged from 347,48mg/Kg to 483,30mg/Kg for Spanish lemon pilot plant juice. The maximum concentration of AIJN is achieved 150,00 and the minimum is 0mg/Kg (Table 1). The interval of ascorbic acid is increased on the comparison with the AIJN. According to our results the range of ascorbic acid has been from 150mg/Kg to 750mg/Kg (Fig. 3).

According to some bibliographic data, intervarietal differentiation has been established by multivariate pattern recognition involving amino acids, flavanone glycosides (Mouly et al., 1994), and flavor constituents (Maccarone et al., 1998). The concentration of amino acids was evaluated to develop a criteria of fruits juices and use it to check variety and the geographical origin of commercial products (fruits juices and beverages) (Wallrauch, S., et al. 1988). The content of amino acids including the data of maximum and minimum concentrations of AIJN is given in Tables 3, 4. The results of our studies show that the aspartic acid, proline, glutamic acid, and asparagine were the principal of amino acids among them in the Spanish lemon juice. The value of aspartic acid achieved the highest value (2,68-4,42Mmol/L) (the maximum concentration of AIJN is 6,02 and the minimum is 2,26), followed by proline (1,2-4,0Mmol/L) (the maximum concentration of AIJN is 6,96 and the minimum is 0,87), followed by glutamic acid (1,27-2,24Mmol/L) (the maximum concentration of AIJN is 2,72 and the minimum is 1,09), followed by asparagine (1,16-2,47Mmol/L) (the maximum concentration of AIJN is 4,55 and the minimum is 0,99). Serine and alanine were considered as the second most predominant group in lemon juice. The Serine value achieved 1,16-1,39Mmol/L (the maximum concentration of AIJN is 3,52 and the minimum is 1,29) and the alanine value achieved 0,72-1,07Mmol/L (the maximum concentration of AIJN is 2,92 and the minimum is 0,90). Amino acids such as arginine, glycine, valine, threonine, isoleucine, leiucine, phenylalanine, lysine, tyrosine, glutamine, histidine, and metionine were present in small amounts. The minimum level of interval of serine, aspartic acid, glutamic acid, alanine, glycine, and threonine is reduced on the comparison with the minimum AIJN (Table 3 and 4). Intervals of valine и leucine are increased on the comparison with the AIJN. Intervals of phenylalanine and tyrosine are increased insignificantly on the comparison with the AIJN.

Results provided important information on physicochemical characteristics of juices (of Spain juice, Italy juice, Australia juice and others) and on how to make the best use of lemon cultivars in the Spain, which is of significance for technological research and processing practice. Although lemon juices are the main commercial product of lemons, only few studies (Moshonas, M. G., et al., 1972; Shaw, P. E., 1991) have been carried out on the lemon juice volatile fraction. Volatile compounds mainly consist of mono- and sesquiterpene hydrocarbons and oxygenated molecules (aldheydes, monoterpene alcohols, and monoterpene esters). Limonene is by far the most abundant monoterpene in all juices (54,67-63,07% of total) (Fig. 4). The content of β-pinene, γ-terpinene, α-pinene, terpinolene, α-thujene, α-terpinene, and trans-β-ocimene ranged, respectively, from 13,5 to15,89%, from 10,2 to 14,14%, from 1,96 to 2,65%, from 0,37 to 0,73%, from 0,42 to 0,65%, from 0,17 to 0,37%, from 0,13 to 0,21% (Fig. 5). β-Bisabolene representing 0,78-1,62% and trans-α- bergamotene representing 0,5-1,05% of the total are the second most important. Oxygenated compounds are mainly aldehydes (nonanal, citronellal, neral, E-citral); monoterpene alcohols (L-linalool, α-terpineol); and esters (neryl acetate, geranyl acetate). Our present studies showed that oxygenated compounds of the lemon juice are at a percentage of about 3,53-5,03% (Table 5).

A reference guideline is a guideline for what is considered as an acceptable juice. Parameters listed under sector “A” are absolute (min. /max.) requirements with respect to the quality of a juice. Values of volatile oils indicated correspond to 0.5 ml/L maximum (AIJN). While the citrus oil exhibits excellent antibacterial ability against various bacteria. Moreover, since the antibacterial agent has the ability to inhibit the growth of dental caries bacteria. Since the antibacterial agent of the present invention also has the ability to inhibit the growth of periodontosis bacteria (Hiramoto et al., 2002).

Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones. A large range of low and high molecular weight plant polyphenolics presenting antioxidant properties has been studied and proposed for protection against lipid oxidation. Other physiological activities of natural antioxidants have been described, such as antibacterial, antiviral, antimutagenic antiallergic, anticarcinogenic effects, antimetastasis activity, platelet aggregation inhibition, blood-pressure increase inhibition, antiulcer activity and anticariogenicity (Andrés Moure., et al., 2001). Thus, flavones that possess antimutagenic activity, flavanones and xanthones, that exhibit antiviral, antimicrobial and anti-inflammatory activities, and isoflavones and coumestans that present important physiological effects in humans, have antioxidant action. The main flavanones are detected in lemon juices (of two clones of “Fino” lemon) with decreasing contents as follows: eriocitrin > hesperidin> diosmetin -6,8-

diglucoside>diosmin. Eriocitrin, hesperidin, diosmetin diglucosides constituted approximately 95% of the total flavonic content present in lemon juices (Elena Gonzalez-Molina, et al., 2008). The content of flavanoid glycosides is strongly influenced by the fruit texture and the technology. Additions, the "genuine" hesperidin content determined by means of HPLC is smaller than the "Davis value" (maximum 1500mg/L) and varies between 200 and 800 mg/L for cloudy juices (AIJN). For clarified products the flavanoids values, especially for hesperidin, are lower. The average concentration of hesperidin of the industry process and the pilot plant process was tested. Hesperidin content were 252,21-399,47 and 352,95-377,35mg/L, in the Spanish industry juice and pilot plant juice, respectively. Higher flavonoid content in lemon juice would improve the intake of these compounds and could increase their absorption in humans.

Di Mauro, A, et al. (Di Mauro, A., et al., 2002) assumed that the characteristic property of the juice should be searched for in the concentration of the minor compound. For example, the concentration of hydroxycinnamic acids (HCA) was evaluated to develop a data bank of Italian juices and use it to check variety and the geographical origins of commercial products. This methodology is useful for characterizing pigmented orange juices, and could be included in the regulations of PGI of Sicilian pigmented orange fruits and for PDO of the corresponding juices. The concentration of cinnamics or benzoics acids can be used to develop a data bank of lemon juices (caffeic and vanillic acids).

The mineral concentration of samples of industry and pilot plant juice, including the maximum and the minimum concentration of AIJN are summarized in Tables 1, 2. Results are presented as average and range (minimum – maximum) values for each of the category tested. Out of these five parameters that were determined in Spanish juices, the all is varied significantly between region of Spain and Australian (Simpkins, W. A., et al., 2000) (Table 1, 2, 6). These were sodium, calcium and phosphorus (higher in Spain samples of the industry process and the pilot plant process), and potassium (lower in Spain samples of the industry process and the pilot plant process), and manganese (lower in Spain samples of the industry process). Range values of Spain samples were higher and larger than its values of AIJN (Table 1, 2). The results showed that potassium was the most abundant mineral (the average concentration 1164,44 - 1399,10mg/Kg and 1241,91-1475,64mg/Kg) in the Spanish juice, followed by calcium (the average concentration 131,12-242,52mg/Kg and 92,37-149,71mg/Kg) and magnesium (the average concentration 87,77-124,27mg/Kg and 87,19-104,03mg/Kg). The average concentration of sodium was 22,09-91,97mg/Kg and 15,43-38,52mg/Kg in the industry juice and pilot plant juice, respectively.

Our objective in this study was to characterize and compare the seasonal change in juice bioactive substances of "Fino" and "Verna" lemons. The database of analytical results (Fig. 4, 5) covers different varieties of Spanish lemon juice, coming from different "Fino" and "Verna" fruits. A gradual increase of volatile compounds in juice with fruit maturity occurred with cultivars in each season. The β-pinene, γ-terpinene, α-pinene, terpinolene, α-thujene, α-terpinene, trans-β-ocimene β-bisabolene, trans-α- bergamotene of Spanish lemon juice during April to June runs between 14,41 and 15,89; 11,07 and 14,14; 2,08 and 2,50; 0,42 and 0,73; 0,46 and 0,65; 0,20 and 0,37; 0,20 and 0,13; 0,84 and 1,62; 0,54 and 1,05, respectively. However, only Limonene is decreased from 63,07to 54,67%. Changes of volatile compounds in juice were significant as a function of fruit maturity. The growing unripe fruit synthesizes high-molecular structures such as proteins, polysaccharides, lipids and flavonoids by carbohydrate metabolism initiated by photosynthesis in the leaves. During ripening, catabolic reactions predominate and the production of volatiles occurs during a short period. In fact, many aroma compounds in fruits and plant materials derive from lipid metabolism. The composition of juice volatile compounds of Spain differs from the composition of juice volatile compounds of Italy. These data could be used for the analytical differentiation of lemon juices.

CONCLUSION

1. The lemon juice differs in its antioxidant capacity depending on the geographical origins.

2. The difference in ascorbic acid equivalent antioxidant capacity of Spanish juice is connected with the change of phytochemical composition in the juice and with the change of the concentration such biofunctional component as ascorbic acid.

3. A difference in ferric reducing antioxidant power is connected with the change of polyphenolic phytochemical composition and of mineral concentrations in the juice.

4. Quantitative results allowed the differentiation of Spain variety geographical origins, in particular, the Mediterranean area from tropical and subtropical areas, using multidimensional analyses of caffeic and vanillic acids contents and antioxidant capacity.

5. As noted earlier, the juice of Spain is contained by 344,85-513,52mg/Kg AA, the juice of China -233,44mg/Kg.

6. The juice of Spain is contained hesperidin 252,21-399,47mg/L, the juice of China-237,96mg/L, while the juice of California -197mg/L.

7. Range values of the mineral concentration of Spain samples were higher and larger than its values of the AIJN.

8. The citric acid/isocitric acid ratio of juices from Spain values in the higher range, 120-350.

9. The minimum level of interval of serine, aspartic acid, glutamic acid, alanine, glycine, and threonine is reduced on the comparison with the minimum AIJN.

10. The interval of valine and leucine are increased on the comparison with the AIJN.

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*Abbreviations: (AIJN), Association Industries of Juice and Nectars Producers; AA, ascorbic acid; AEAC, ascorbic acid equivalent antioxidant capacity; FGs, flavanone glycosides; FRAP, ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl; (PGI), Protected Geographical Indication; (PDO), Protected Designation of Origin; Av, average.

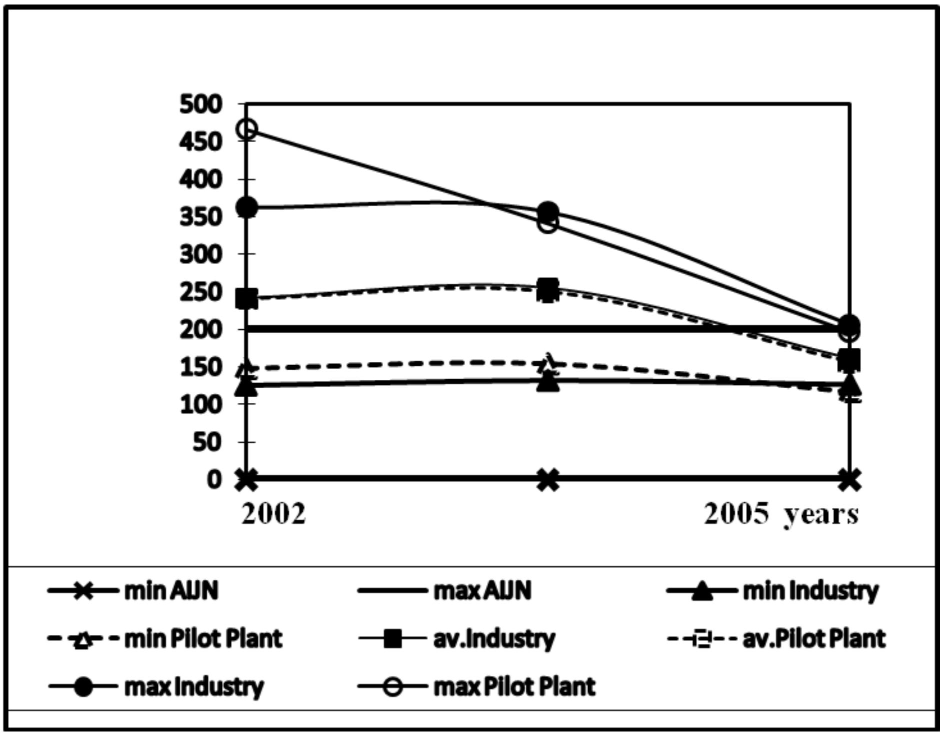

Figure 1. Comparison of seasonal variation in citric acid/isocitric acid ratio for lemon juice of Spain (2002-2005): minimum, average and maximum values of the industrial juice; minimum, average and maximum values of the pilot plant juice; minimum and maximum values "AIJN"; min. AIJN=0.

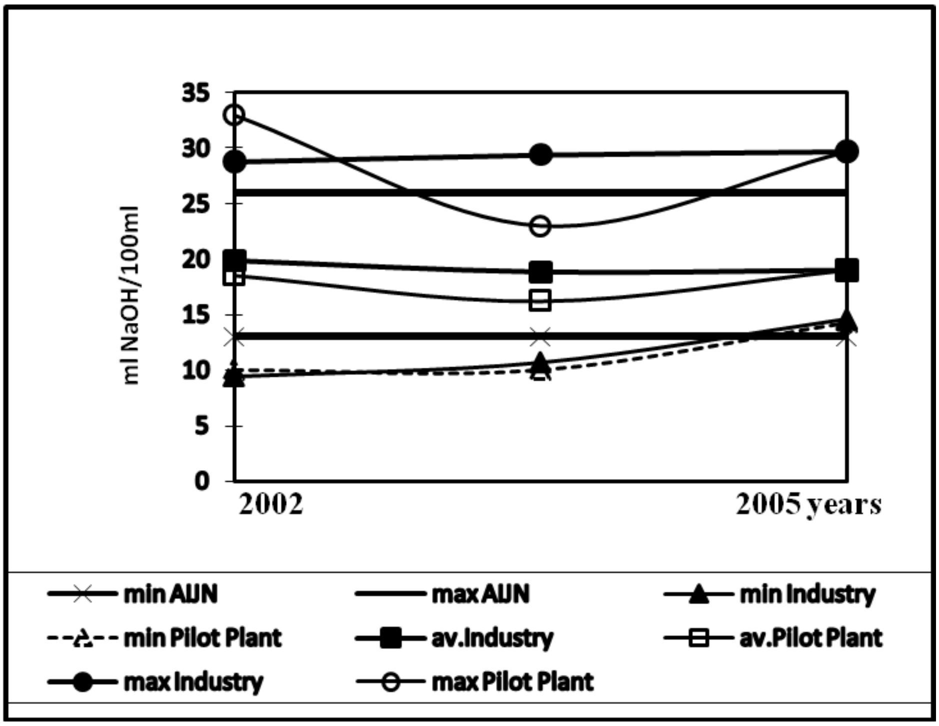

Figure 2. Comparison of seasonal variation in Formol number for lemon juice of Spain (2002-2005): minimum, average and maximum values of the industrial juice; minimum, average and maximum values of the pilot plant juice; minimum and maximum values "AIJN".

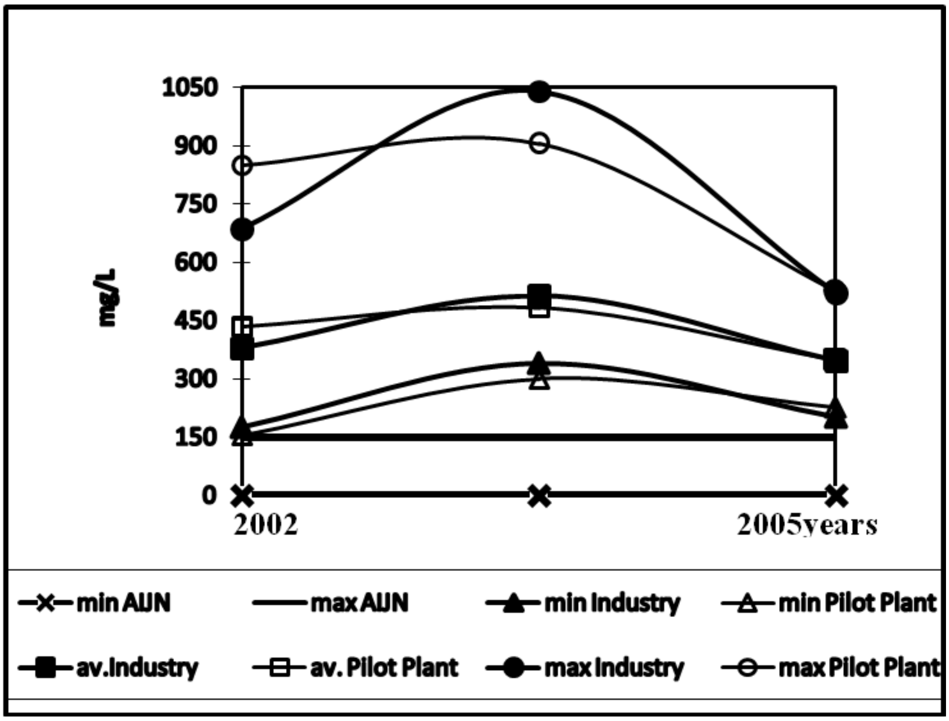

Figure 3. Comparison of seasonal variation in ascorbic acid for lemon juice of Spain (2002-2005): minimum, average and maximum values of the industrial juice;

minimum, average and maximum values of the pilot plant juice; minimum and maximum values "AIJN"; min. AIJN=0.

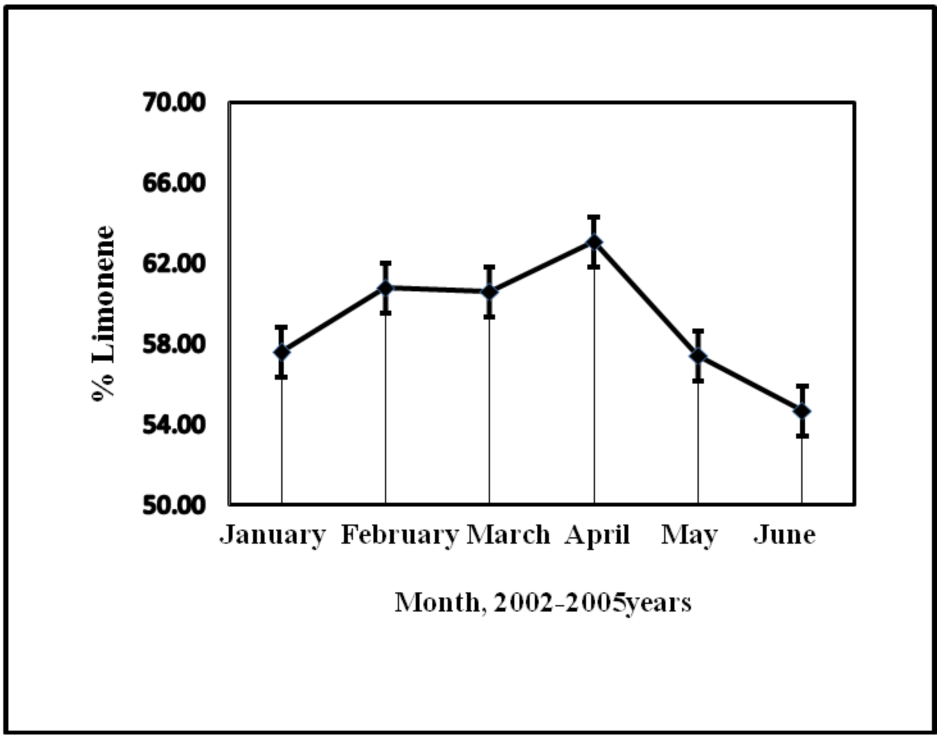

Figure 4. Comparison of seasonal variation in limonene for Spanish juice of lemon "Fino" (January, February, March, April) and lemon "Verna" (May, June).

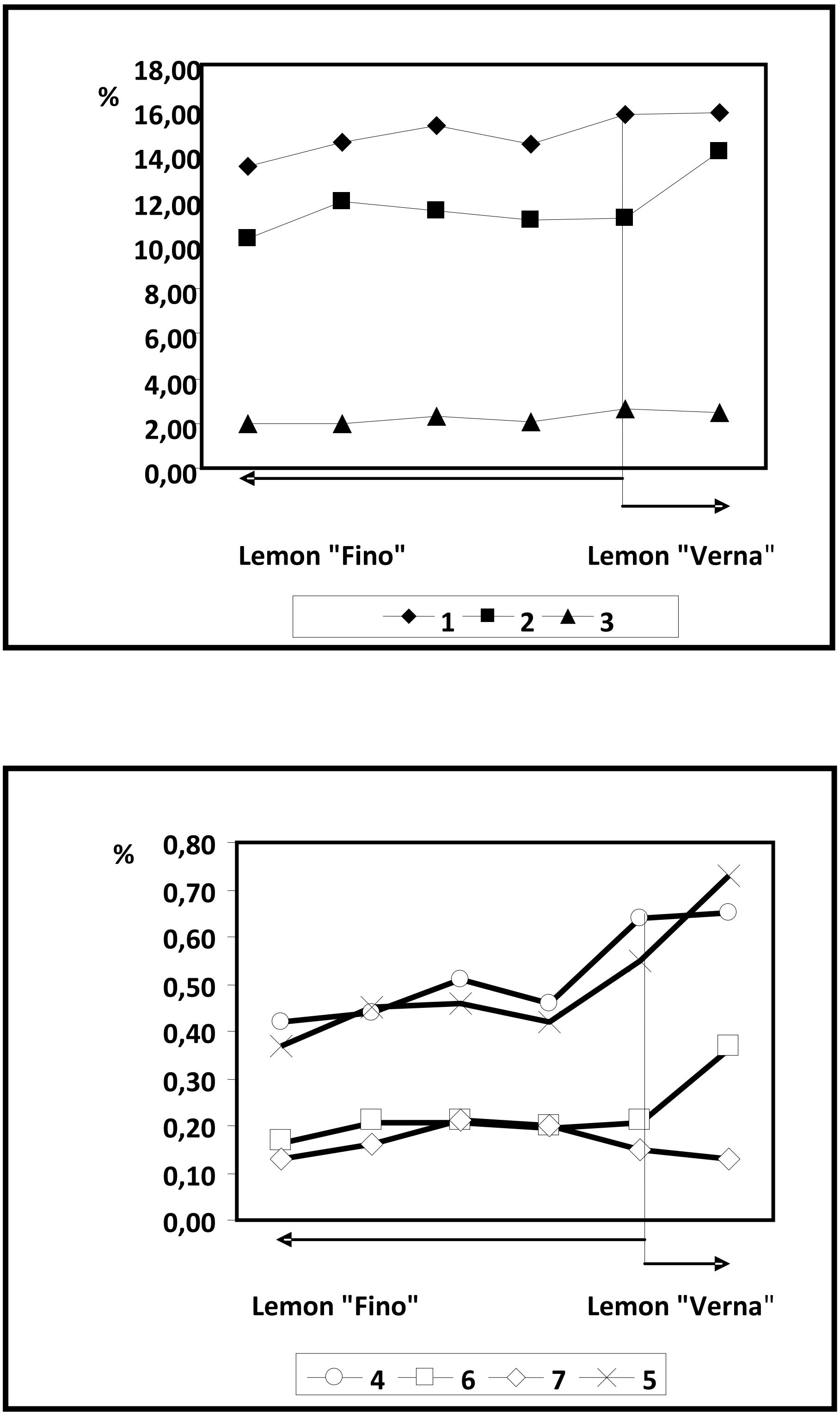

Figure 5. Comparison of seasonal variation in volatile contents for Spanish juice of lemon “Fino”(January, February, March, April) and lemon “Verna” (May, June): 1- β-pinene, 2- γ-terpinene, 3- α-pinene , 4-terpinolene, 5-α-thujene, 6-α-terpinene and -trans-β-ocimene.

Table 1. Seasonal quantitative criteria changes in the industrial juice (° Brix a 20°C - 8, 0; lactic acid –not detected).

Parameters	Units	AIJN		Season 2002-2003			Season 2003-2004			Season 2004-2005		
		Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max
Total acid	g /Kg[1]	-	-	55,06	39,92	67,49	54,06	44,83	78,06	48,62	38,40	59,80
Volatile acid	g /Kg[2]	0	0,40	0,01	0,00	0,05	0,01	0,00	0,04	0,18	0,05	0,37
Formol number	[3] ml NaOH/100	13	26	19,84	9,41	28,75	18,85	10,67	29,39	19,01	14,60	29,79
Total phosphorus	mg/Kg	250	450	358,75	238,51	606,42	287,28	235,64	355,54	295,02	164,80	406,70
Ashes	g/L	2,2	4,3	2,81	1,39	3,56	2,88	1,40	3,88	3,22	2,40	3,70
Sodium	mg/Kg	0	30	22,09	6,54	71,14	91,97	6,80	225,28	35,35	2,80	105,30
Potassium	mg/Kg	1100	2000	1475,64	1101,12	2491,43	1241,91	398,51	2152,76	1294,7	1075,2	1602,8
Calcium	mg/Kg	45	160	131,12	56,44	605,71	242,52	62,90	655,85	141,57	65,20	280,40
Magnesium	mg/Kg	70	120	87,77	60,99	120,57	124,27	56,99	429,08	106,92	77,10	127,50
Ascorbic acid	mg/Kg	0	150	378,82	175,07	685,71	513,52	338,50	1039,09	344,85	200,00	520,00
Citric acid	g/Kg	45	63	48,26	34,18	57,98	54,06	44,83	78,06	49,30	36,00	59,40
Malic acid	g/Kg	1	7,5	1,22	0,65	2,14	1,36	0,18	2,15	2,68	1,00	5,00
Isocitric acid	mg/Kg	230	500	208	138	329	254	172	556	308	219	396
Glucose	g/L	3,0	12,0	8,47	5,76	15,54	7,63	0,96	14,40	8,26	3,60	10,80
Fructose	g/L	3,0	11,0	8,03	5,52	15,77	7,27	0,96	15,20	7,45	3,20	10,70
Sucrose	g/L	0	7	4,00	1,87	10,50	3,05	0,53	8,00	3,42	1,30	6,40

[1]- g Citric Acid/Kg
[2]- g Acetic Acid/Kg
[3]- ml NaOH 0,1Mol /100

Table 2. Seasonal quantitative criteria changes in the plant pilot juice (lactic acid –not detected).

Parameters	Units	Season 2002-2003			Season 2003-2004			Season 2004-2005		
		Av	Min	Max	Av	Min	Max	Av	Min	Max
Acid total	g /Kg[1]	52,91	35,60	104,90	54,61	35,10	65,60	51,24	40,10	62,20
Volatile acids as acetic acid	g /Kg[2]	0,04	0,01	0,18	0,03	0,02	0,05	0,20	0,09	0,38
Formol number	[3] ml NaOH/100	18,56	10,00	33,00	16,22	10,00	23,00	17,91	14,20	28,70
°Brix	20°C	8,1	5	12,5	7,9	6	10	7,8	6,8	8,8
Total phosphorus	mg/Kg	348,54	130,79	694,67	293,47	201,50	437,60	276,41	182,70	353,80
Ashes	g/L	2,54	1,20	5,00	2,30	1,70	3,20	3,01	1,90	4,00
Sodium	mg/Kg	15,43	5,60	38,47	38,52	8,40	109,60	15,57	3,0	75,60
Potassium	mg/Kg	1399,1	801,60	1953,1	1164,4	673,60	2165,8	1281,4	1015,3	1676,4
Calcium	mg/Kg	98,01	51,50	137,70	149,71	53,40	401,30	92,37	46,80	153,90
Magnesium	mg/Kg	87,19	46,70	131,20	88,88	37,50	230,80	104,03	74,00	136,70
Ascorbic acid	mg/Kg	435,34	154,20	850,00	483,30	300,20	904,60	347,48	224,60	524,70
Citric acid	g/Kg	51,05	35,06	104,10	54,61	35,10	65,60	51,21	41,00	59,60
Malic acid	g/Kg	1,47	1,00	7,01	1,42	1,01	2,15	2,53	1,00	5,00
Isocitric acid	mg/Kg	226	114	573	252	184	406	332	254	456
Glucose	g/L	8,13	4,70	15,70	8,16	5,00	11,80	7,52	3,00	13,00
Fructose	g/L	7,48	4,60	13,60	7,48	4,10	11,40	6,94	3,40	10,40
Sucrose	g/L	4,80	1,20	9,00	5,18	1,90	15,00	3,60	1,00	6,0

[1]- g Citric Acid/Kg
[2]- g Acetic Acid/Kg
[3]- ml NaOH 0,1Mol /100

Table 3. Changes in the amino acid content (Mmol/L) in industrial juice as a function of fruit ripening.

**Amino acid	Season 2002-2003			Season 2003-2004			Season 2004-2005		
	Av	Min	Max	Av	Min	Max	Av	Min	Max
Aspartic acid	1,35	1,10	1,76	3,11	3,00	3,25	4,45	1,98	5,13
Threonine	0,05	0,04	0,07	0,13	0,09	0,22	0,15	0,12	0,19
Serine	0,71	0,56	0,92	1,21	1,10	1,30	1,34	1,05	1,92
Asparagine	0,93	0,74	1,27	1,14	1,00	1,25	2,47	1,11	3,08
Glutamic acid	0,62	0,47	0,86	1.41	1,30	1,60	2,15	1,98	2,37
Glutamine	0,06	0,03	0,10	0,00	0,00	0,01	0,19	0,11	0,29
Proline	1,49	1,16	1,87	1,47	1,40	1,60	4,15	3,30	5,26
Glycine	0,06	0,03	0,08	0,14	0,10	0,20	0,09	0,06	0,14
Alanine	0,61	0,45	0,75	1,05	0,95	1,20	0,81	0,56	1,08
Valine	0,07	0,04	0,09	0,22	0,15	0,30	0,09	0,07	0,12
Methionine	0,01	0,00	0,02	0,00	0,00	0,00	0,02	0,00	0,03
Isoleucine	0,02	0,01	0,03	0,05	0,04	0,07	0,05	0,03	0,06
Leucine	0,02	0,01	0,03	0,06	0,05	0,07	0,05	0,02	0,07
Tyrosine	0,01	0,00	0,02	0,00	0,00	0,00	0,04	0,03	0,05
Phenylalanine	0,06	0,04	0,07	0,13	0,09	0,20	0,19	0,09	0,27
Lysine	0,04	0,02	0,05	0,13	0,08	0,20	0,10	0,08	0,17
Histidine	0,02	0,01	0,03	0,00	0,00	0,01	0,04	0,03	0,05
Arginine	0,35	0,25	0,51	0,31	0,20	0,40	0,35	0,25	0,51

Table 4. Changes in the amino acid content (Mmol/L) in pilot plant juice as a function of fruit ripening and the minimum, the maximum value of "AIJN".

*Amino acid	AIJN		Season 2002-2003			Season 2003-2004			Season 2004-2005		
	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max
Asp	2,26	6,02	2,68	2,00	4,00	3,12	3,01	3,15	4,42	2,09	5,36
Thr	0,08	0,25	0,09	0,08	0,11	0,13	0,09	0,22	0,15	0,10	0,20
Ser	1,29	3,52	1,16	0,09	2,00	1,21	1,12	1,30	1,39	1,11	1,84
Asn	0,99	4,55	1,23	0,95	2,00	1,16	1,05	1,22	2,47	1,29	2,99
Glu	1,09	2,72	1,27	1,01	1,75	1,40	1,30	1,55	2,24	1,90	2,89
Gln	-	0.31	0,01	0,00	0,05	0,00	0,00	0,01	0,17	0,08	0,22
Pro	0,87	6,96	1,20	0,86	2,00	1,46	1,40	1,55	4,00	3,14	5,23
Gly	0,09	0,33	0,12	0,09	0,18	0,14	0,09	0,22	0,09	0,05	0,12
Ala	0,90	2,92	1,07	0,90	1,80	1,06	0,99	1,25	0,72	0,61	0,85
Val	0,07	0,30	0,10	0,07	0,21	0,23	0,17	0,35	0,10	0,06	0,17
Met	-	0,03	0,00	0,00	0,01	0,00	0,00	0,02	0,02	0,01	0,03
Iso	0,02	0,08	0,03	0,02	0,05	0,05	0,05	0,06	0,05	0,03	0,06
Leu	0,02	0,08	0,03	0,02	0,05	0,06	0,05	0,07	0,05	0,03	0,07
Tyr	-	0,04	0,01	0,00	0,02	0,00	0,00	0,01	0,04	0,03	0,05
Phe	0,05	0,24	0,08	0,03	0,20	0,14	0,10	0,25	0,18	0,09	0,24
Lys	0,03	0,14	0,05	0,03	0,10	0,13	0,09	0,20	0,09	0,07	0,13
His	-	0,07	0,02	0.00	0,04	0,01	0,00	0,02	0,04	0,02	0,05
Arg	-	0,58	0,29	0,20	0,36	0,33	0,30	0,45	0,29	0,20	0,36

Table 5. Oxygenated compounds. Season 2003-2004 years.

Oxygenated compounds, %	January	February	March	April	May	June
Nonanal	0,09	0,09	0,09	0,08	0,13	0,11
Citronellal	0,11	0,13	0,12	0,12	0,20	0,18
Neral	0,84	0,90	0,86	0,80	0,93	0,73
E-Citral	1,49	1,64	1,51	1,39	1,61	1,19
Linalool	0,09	0,09	0,07	0,08	0,14	0,14
Terpineol	0,16	0,18	0,14	0,18	0,22	0,27
Neryl acetate	0,50	0,58	0,50	0,50	1,02	0,91
Geranyl acetate	0,33	0,41	0,39	0,38	0,78	1,04
Sum	3,61	4,02	3,68	3,53	5,03	4,57

Table 6. Elemental composition (mg/Kg) of fresh juice Australian at natural °Brix and of Australian Prime concentrate reconstituted to 10°Brix.

Element	Fresh juice		Prime concentrate	
	Min. value	Max. value	Min. value	Max. value
Phosphorus	78	270	98,1	242
Sodium	0,58	71	3,5	94,7
Potassium	777	2345	1061	2250
Calcium	35	160	37,8	125
Magnesium	55,1	170	41,1	153