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Influence of processing variables on some characteristics of nocino liqueur

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Abstract

The production of nocino, an after-dinner liqueur of Celtic origin, is connected with ancient superstitions and legend. The date upon which the walnuts should be gathered (the night of 24 June) seems to be one of the few universally accepted features of the production process. The aim of this study was to investigate the influence of the relative ripeness of the walnuts, together with the effect of temperature and the length of steeping on the phenolic composition and the antioxidant power of nocino. Three different batches of unripe walnuts gathered from a single nut tree were used to produce 18 samples of alcoholic infusion. The steeping process was carried out at 20 or 40 °C, for 15, 40 or 90 days. The results revealed that the highest content of phenolic substances – and consequently the highest antioxidant power – was obtained with the least ripe batch of walnuts. On the other hand, temperature and length of steeping have little effect on the phenolic composition of nocino.

Keywords: Antioxidant; DPPH; Nocino liqueur; Phenols; Production process; Walnut

1. Introduction

Nocino is an after-dinner liqueur of Celtic origin, which is very well-known and appreciated in Italy and other countries. Although it is one of Italy's typical products, it has never been the object of scientific research, either as regards its composition or, even less, the production process. The little information available on walnut liqueur concerns its history and associated folklore or the suggested recipes for household preparation (Bergonzini, 1978).

The production of nocino is connected with ancient superstitions and legend. Indeed, tradition has it that the unripe, green walnuts, intended to be used for producing the liqueur, should be collected on the night of 24 June, the day dedicated to Saint John the Baptist, which coincides with the end of the celebrations for the summer solstice. It was held that herbs and nuts gathered during this period were influenced by the change in the sun's ecliptic and the growth cycle in plants, and would thus be particularly purifying.

The date upon which the walnuts should be gathered seems to be one of the few universally accepted features of the production process for nocino. On the other hand, the recipes used and the methods of preparation are many and various. When collected, the walnuts still have a green hull and the endocarp is developed, but has not hardened. The whole nuts are washed, quartered and left to steep in food-grade ethanol in glass or enamelled pottery containers. The ratio of walnuts to alcohol should be about 1:2, so as to obtain an alcohol content of around 60% v/v. In industrial production, the mass is left to brew in silos, protected from the direct light of the sun, for at least 4 months, and constantly stirred. At the end of the brewing period, the steeped mass is pressed to separate the liquid phase from the solid matter and left

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to age between 6 months and 4 years. It must not be pressed too vigorously, otherwise unpleasant-tasting substances may pass into the liquid. After aging, a sugar syrup is added to the brew thus obtained, so the final alcohol content is around 38–43% v/v. Also natural herbs, spices and flavourings could be added, such as, for example, cloves, cinnamon, coriander, coffee beans and lemon zest, which differentiate the various formulations. The liquor thus obtained is filtered prior to bottling (technical document, Il Mallo, Pozza di Maranello, Modena, Italy).

The main variables in the process – apart, naturally, from the formulation used – are temperature, the length of steeping and aging time. Moreover, setting aside popular beliefs, the ripeness of the walnuts can be considered an important variable.

A previous study (Alamprese, Pompei, & Scaramuzzi, 2004) highlighted the fact that nocino is characterized by a significant content of phenolic substances with a high antioxidant power. However, the characteristics of the liqueurs sold under the name of ''nocino'' were found to vary considerably. It was, moreover, observed that aging did not lead to a significant reduction in polyphenol content and thus in the antioxidant power of the liqueur.

The aim of this study was to investigate the influence of the relative ripeness of the walnuts, together with the effect of temperature and the length of steeping, on the phenolic composition and the antioxidant properties of nocino, in order to extract useful information with a view to standardizing the most interesting characteristics of this liqueur.

2. Materials and methods

2.1. Materials

Three batches of unripe walnuts were gathered from a single nut tree (*Juglans regia*, cv. Noce di Sorrento):

- batch 13/06: gathered on 13 June
- batch 24/06: gathered on 24 June
- batch 04/07: gathered on 4 July

Each batch was made up of around 80 walnuts of various sizes; the average weights of nuts belonging to the three different batches were 27.40 ± 0.26, 34.23 ± 0.21 and 38.98 ± 0.49 g, respectively.

The three batches were characterized by analysis of the moisture content, ash and firmness. The phenol composition and the antioxidant properties were evaluated on an ethanolic extract: 100 g of walnuts were washed, chopped and blended for 3 min in a mixture of 96% (v/v) ethanol (BDH Laboratories Supplies, Poole, UK) and distilled water, introduced in such proportions as to obtain around 600 ml of extract in ethanol at 80% v/v. In order to calculate the exact quantities of ethanol and water required, the water content of the walnuts was taken into account. This was determined by means of a moisture content analysis for each batch. The samples were kept in the dark, at 20 °C, for 24 h. The hydro-alcohol phase was then retrieved by means of a filtration using Whatman no. 1 filter papers (Whatman International Ltd., Maidstone, UK) and restored to its initial volume (600 ml) with 80% v/v ethanol. The ethanolic extract aliquot set aside for analyses was poured into 15 ml test tubes, saturated with nitrogen and kept in the dark at 20 °C.

The three batches of unripe walnuts produced 18 samples of alcoholic infusion, combining different temperatures and times of steeping. Approximately 270 g walnuts, cut into quarters, were put in a glass container, together with 500 ml of food-grade ethanol (95% v/v). The steeping process was carried out in the dark, at 20 or 40 °C, for 15, 40 or 90 days. The glass containers were periodically shaken. At the end of this phase, the liquid was separated, by filtering through paper filters, and dispensed into 15 ml tubes, saturated with nitrogen and stored at 20 °C, in the dark. The samples were identified by the gathering date of the corresponding batch of walnuts, followed by the steeping time expressed in days (15 d, 40 d, 90 d).

2.2. Walnut firmness

The firmness of the walnuts was evaluated by means of penetration tests, carried out with an Instron Universal Testing Machine (mod. 4301, Instron Ltd., High Wycombe, UK), connected to a personal computer which manages the instrument by means of dedicated software (Series IX Automated Material Testing System software, version 7.50.00, Instron Corp., 1998). The tests were carried out using a steel probe with a 4-mm diameter and a 45° angle point, at a penetration speed of 20 mm/min, with a 1-kN load cell. Each walnut was positioned on the equipment lengthwise and in such a way that it was not perforated along the seam uniting the two halves of the shell. Results are expressed in terms of load at the 1st and 2nd peak (N) and energy (J) and are the average of 20 determinations.

2.3. Walnut moisture and ash contents

The moisture and ash contents of the unripe walnuts were determined by gravimetric methods, following AOAC Official Method Nos. 925.40 and 950.49, respectively (AOAC, 1995). Samples were previously minced in a Waring Blender (mod. 32B/79, Dinamics Corporation of America, New Hartford, USA), for 3 min, at the minimum frequency. Results are reported as g/100 g.

2.4. Phenolic composition

Total phenols were determined using the Folin–Ciocalteau reagent according to Singleton and Rossi (1965). Also, non-tannin phenolics were analysed by the Folin–Ciocalteau method, but with prior precipitation of tannins with cinchonine sulphate (Peri & Pompei, 1971). Total tannins were calculated as the difference between total phenols and non-tannin phenolics. Results are expressed as milligrammes of catechin/100 g for walnuts, and as milligrammes of catechin/l for infusions.

2.5. pH

The pH of the infusions was measured using a pH-meter mod. pH M62 (Radiometer, Copenhagen, Denmark).

2.6. Sugar and ethanol contents

Sugar and ethanol contents were determined by HPLC, following the method reported by Yuan and Chen (1999), modified by Alamprese et al. (2004). Results are expressed as g/100 ml for sugars and as % v/v for ethanol.

2.7. Antioxidant activity

Antioxidant activity was measured by two methods: the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\cdot}$) method (Brand-Williams, Cuvelier, & Berset, 1994) and the electrochemical method suggested by Mannino, Brenna, Buratti, and Cosio (1998). Both the analyses were performed as reported by Alamprese et al. (2004). In the case of the DPPH$^{\cdot}$ method, results are reported as $1/I_{50}$, where I_{50} was the amount of original infusion sample (in microlitres) required to lower the initial DPPH$^{\cdot}$ concentration by 50% and was extrapolated from a dose-response curve. For the electrochemical method results are expressed as trolox equivalents (TE), reported as micromoles of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in 100 ml, and calculated with respect to a calibration curve of trolox standard solutions in a concentration range of 2–7 mg/l. To relate results to 100 g of walnuts, data obtained from ethanolic extracts were multiplied by 0.6.

Except where otherwise stated, all the above analyses were carried out at least in duplicate.

2.8. Statistical analyses

One way ANOVA and Pearson correlation matrix were calculated using Systat 5.03 for Windows (Systat, Inc., Evanston, IL, USA).

3. Results and discussion

3.1. Characterization of walnuts

The values for moisture and ash contents of the walnuts from the batches gathered on the three different dates (13 June, 24 June, 4 July) are reported in Table 1. As expected, the moisture content of the walnuts from the three batches fell slightly as ripening progressed, although this decrease was not significant ($P > 0.05$). The average value for the three batches proved to be about 85.5 g/100 g. On the other hand, the ash content increased significantly ($P < 0.01$) between the first and the second gathering, and then stabilized at around 0.8 g/100 g.

The penetration tests for the unripe walnuts provided the data for the rheological parameters reported in Table 1. The loads at the first and the second peak show the maximum resistance to the penetration of the steel piston by the upper and lower halves of the shell, respectively. The energy required corresponds to the area subtended by the penetration curve, in an interval of between 2 and 45 mm. From the data reported in the Table it can be noted that, as the walnuts ripened, the hardness of the shell increased considerably: indeed, the walnuts from the batch gathered latest had peak load values three times higher than those from the first batch. The average firmness of the walnuts, evaluated in terms of energy, showed a modest but significant increase ($P < 0.01$) between the first and the second batch gathered, while it doubled between the first and the third batch gathered ($P < 0.001$).

The concentrations of glucose and fructose in the walnuts, evaluated on the ethanolic extract, proved to be below detectable limits for the method utilized (21.1 and 18.0 mg/l, respectively).

Table 2 shows the pH values, phenolic composition and antioxidant activity of the unripe walnuts evaluated on the ethanolic extract at 80% v/v. The pH values of the ethanolic extracts showed a slight increase as the nuts progressively ripened, following the trend for ash content.

According to the study by Anderson et al. (2001), the phenolic substances present in the kernel of the ripe nut consist predominantly of non-flavonoid phenols, such as ellagitannins and similar compounds (hydrolizable tannins). However, there is no information to be found in the literature about the phenolic substances in the hull of the unripe nuts.

The results obtained in this study show an average total phenol content for the three batches of approximately 659 mg catechin/100 g. The maximum value was found in the least ripe batch of walnuts: those gathered at the earliest date (batch 13/06). The total phenol content decreased as the walnuts ripened, even though the difference was truly significant ($P < 0.05$) only when

Table 1
Moisture, ash content and rheological parameters (average ± s.d.) of the three batches of walnuts

Sample	Moisture (g/100 g)	Ash (g/100 g)	Rheological parameters		
			Load at 1st peak (N)	Load at 2nd peak (N)	Energy (J)
13/06	87.36 ± 0.12^{a}	0.47 ± 0.01^{a}	38.9 ± 2.8^{a}	56.2 ± 4.1^{a}	1.23 ± 0.13^{a}
24/06	85.35 ± 1.55^{a}	0.83 ± 0.07^{b}	61.3 ± 7.7^{b}	80.8 ± 11.2^{b}	1.55 ± 0.15^{b}
04/07	83.83 ± 0.23^{a}	0.80 ± 0.01^{b}	130.7 ± 20.1^{c}	174.2 ± 18.2^{c}	2.65 ± 0.28^{c}

^{a,b,c} Averages in the same column without a common subscript are significantly different ($P < 0.05$).

Table 2
pH values, phenolic composition and antioxidant activity (average ± s.d.) of the unripe walnuts

Sample	pH	Total phenols (mg/100 g)	Non-tannin phenolics (mg/100 g)	Total tannins (mg/100 g)	$1/I_{50}$ (μg^{-1})	TE (μmol trolox/100 g)
13/06	4.92	816 ± 73^{b}	324 ± 23^{a}	492	0.90 ± 0.01^{b}	5.7 ± 0.7^{a}
24/06	5.10	626 ± 37^{ab}	278 ± 2^{a}	348	1.07 ± 0.01^{c}	6.4 ± 0.6^{b}
04/07	5.16	536 ± 17^{a}	287 ± 2^{a}	249	0.81 ± 0.01^{a}	5.4 ± 0.2^{a}

^{a,b,c} Averages in the same column without a common subscript are significantly different ($P < 0.05$).

the first and the third gatherings were compared. There did not prove to be any significant differences between the non-tannin phenol content in the three batches, which had an average value of 296 mg catechin/100 g. Total tannins, as already observed for total phenols, decreased with the increase in walnut ripeness, both in absolute terms and as a percentage compared with total phenols (falling from 60% to 46%).

The maximum value for antioxidant activity, evaluated on the ethanolic extracts, was recorded for batch 24/06. This result was obtained by both assessment methods.

3.2. Characterization of alcoholic infusions

The average alcohol content for the infusions produced proved to be 65.6 ± 1.6% v/v. As regards sugars, only fructose was present in detectable quantities, at an average level of 0.57 ± 0.04 g/100 ml.

Tables 3 and 4 show the pH values and the phenolic composition of the infusions produced from the three batches of walnuts with different steeping times, at 20 and 40 °C, respectively.

Both in the samples produced at 20 °C and in those produced at 40 °C, the pH increased according to the duration of infusion. The average pH value for the infusions prepared with the least ripe walnuts (batch 13/06) was lower than that of the other six infusions, both at 20 °C (average pH 4.77) and at 40 °C (average pH 4.78), in line with the results obtained for the walnuts themselves. However, the temperature of the infusion did not seem to affect this characteristic systematically.

The total phenol content in the infusions showed a tendency to decrease with storage time, after the walnuts were removed. This decrease was observed despite the fact that the samples had been divided up and placed in 15 ml test tubes, saturated with nitrogen and kept at 20 °C, away from light. By way of example, Fig. 1 shows the trend in total phenol contents of the infusions prepared with batch 13/06 at 40 °C, recorded up to approximately 100 days of storage. For all three walnut batches, and at both infusion temperatures, the greatest decrease in total phenols was observed in the samples produced with a 15-day infusion period. In these samples, the average reduction in total phenols was approximately 42%. The lowest decrease (of around 7%) was

Table 3
pH values and phenolic composition (average ± s.d.) of infusion samples produced at 20 °C

Sample	pH	Total phenols (mg/l)	Non-tannin phenolics (mg/l)	Total tannins (mg/l)
13/06-15 d	4.71	2705 ± 161^{aA}	1491 ± 21^{aB}	1214
13/06-40 d	4.79	2546 ± 64^{aB}	1173 ± 23^{aB}	1455
13/06-90 d	4.80	3478 ± 64^{bB}	2111 ± 28^{bC}	1367
24/06-15 d	4.79	1898 ± 209^{aB}	851 ± 72^{aA}	1047
24/06-40 d	5.00	2114 ± 129^{abB}	1248 ± 26^{bB}	867
24/06-90 d	5.08	2694 ± 80^{bA}	1453 ± 54^{bB}	1241
04/07-15 d	4.83	1182 ± 1^{aC}	786 ± 36^{aA}	396
04/07-40 d	4.95	1330 ± 177^{aA}	1064 ± 8^{bA}	266
04/07-90 d	5.04	2533 ± 141^{bA}	1120 ± 10^{bA}	1412

^{a,b,c} Averages with different letters indicate significant differences ($P < 0.05$) between samples produced from the same walnut batch.
^{A,B,C} Averages with different letters indicate significant differences ($P < 0.05$) between samples produced with the same steeping length.

Table 4
pH values and phenolic composition (average ± s.d.) of infusion samples produced at 40 °C

Sample	pH	Total phenols (mg/l)	Non-tannin phenolics (mg/l)	Total tannins (mg/l)
13/06-15 d	4.79	2035 ± 145^{aA}	1180 ± 13^{aB}	871
13/06-40 d	4.69	2830 ± 80^{bB}	1229 ± 51^{aA}	1601
13/06-90 d	4.85	3205 ± 1^{bA}	1862 ± 15^{bB}	1343
24/06-15 d	4.88	2228 ± 96^{aA}	968 ± 26^{aA}	1260
24/06-40 d	5.00	2114 ± 96^{aA}	1271 ± 28^{bA}	843
24/06-90 d	5.12	2751 ± 482^{aA}	1670 ± 103^{cB}	1081
04/07-15 d	4.99	2364 ± 264^{aA}	1149 ± 31^{aB}	1215
04/07-40 d	4.93	1887 ± 96^{aA}	1158 ± 3^{aA}	729
04/07-90 d	5.00	2560 ± 64^{aA}	1226 ± 26^{aA}	1334

[a,b,c] Averages with different letters indicate significant differences ($P < 0.05$) between samples produced from the same walnut batch.
[A,B,C] Averages with different letters indicate significant differences ($P < 0.05$) between samples produced with the same steeping length.

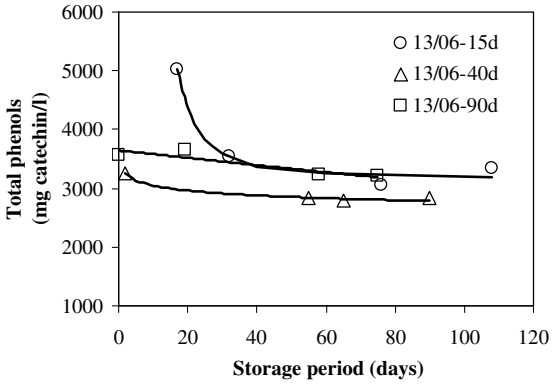

Fig. 1. Trend in total phenol content of the infusions prepared with batch 13/06 at 40 °C.

observed in the samples produced with a 90-day infusion period. This difference could be explained by the fact that, with a longer infusion time, the substances that are more easily oxidizable, or polymerizable, precipitate and are thus no longer detectable when one separates the infusion from the walnuts. On the other hand, when the walnuts are steeped for a short time, these substances are present in the infusion when it has just been separated from the walnuts, but they polymerize and/or precipitate during the storage of the infusion. Moreover, it must be taken into account that polymerized substances react to a lesser extent with the Folin–Ciocalteau reagent, thus giving rise to a lower analytical response. However, in all cases, the phenol content showed a tendency to stabilize after around 80 days of storage. We therefore decided to carry out all the characterization analyses on the infusions when they had already stabilized and after filtration through a HA 0.45 μm membrane (Millipore Co., Milford, MA, USA), so as to be able to compare the various samples, irrespectively of when the analysis was carried out. HA membranes are mixtures of cellulose acetate and cellulose nitrate, used to remove particles from solutions before analyses.

From the data reported in Tables 3 and 4 it can be observed that, after 15 days of steeping at 20 °C, the greatest quantities of phenols were present in infusions produced with the least ripe walnuts (sample 13/06-15 d). Actually, walnuts gathered on 13 June showed the highest total phenol content (Table 2). At 40 °C, however, the total phenol content after 15 days of infusion proved to be greater in the samples produced using the nuts collected at the latest date (sample 04/07-15 d). Extending the steeping time to 90 days resulted in a significant increase ($P < 0.05$) in total phenols in the infusions produced at 20 °C, whereas in the case of steeping at 40 °C, the increase proved to be significant only in the case of batch 13/06. The differences between the infusions produced at 20 °C and those produced at 40 °C, taken from the same batch and steeped for the same length of time, proved to be significant ($P < 0.05$) only for samples 13/06-15 d, 13/06-90 d and 04/07-15 d, which revealed a higher total phenol content in the case of infusion at 20 °C.

On average, about 65% of walnut total phenols was found in the corresponding stabilized infusions.

Although they were produced using the same formulation as the samples of aged, home-made nocino investigated in the previous study, the samples of infusions in this study had a total phenol content which was, on average, lower (by about 45%), taking into account the dilution with sugar syrup which they should normally undergo. This allows us to determine another important variable, even though this is not investigated here: the cultivar of the walnuts used in the preparation of the liqueur.

The non-tannin phenolics, similarly to total phenols, showed a tendency to increase as the steeping time lengthened. The greatest quantity was found in the infusion produced with batch 13/06 and 90 days of brewing time at 20 °C. A higher steeping temperature (40 °C) allowed us to obtain a significantly higher quantity ($P < 0.05$) of non-tannin phenolics only in the case of the infusions prepared with walnut batch 04/07.

As far as total tannin content is concerned, with the same collection date and steeping temperature, no systematic trend was found in terms of infusion time. This might be attributable to subsequent polymerization phenomena and hydrolysis of the tannins. In general, however, total tannins amount to approximately 45% of total phenols, without any substantial difference between the different infusion times and temperatures.

Table 5 shows the results of the analyses of antioxidant activity for the infusions produced at 20 and at 40 °C. Both analytical methods used showed that, with the increase in ripeness of the walnuts, the antioxidant power of the infusion obtained decreases significantly. The influence of time and extraction temperature on antioxidant activity generally appeared to be negligible and non-systematic.

The maximum value for antioxidant power was observed in infusion 13/06-90 d prepared at 20 °C, whereas the minimum was found in sample 04/07-15 d produced at 20 °C. There was a drop in antioxidant power between the first and the last gathering of walnuts, irrespective of steeping time and temperature, equal to 24% if measured by the DPPH· method, and equal to 55% if measured using the electrochemical method.

Utilizing either method, there proved to be a high correlation ($P < 0.001$) between antioxidant power and all three of the phenolic classes analyzed, confirming the observations made by Alamprese et al. (2004).

Combining the data obtained in this study and those reported in the previous research (Alamprese et al., 2004), there proved to be a high correlation between results obtained using DPPH· and those obtained by the electrochemical method ($P < 0.001$) up to values of $1/I_{50}$ of approximately 0.6. Indeed, in Fig. 2 it can be noted that figures above this value of $1/I_{50}$ do not fall within the linear correlation. This could be attributed to a non-linear response in the DPPH· method for high values of antioxidant power.

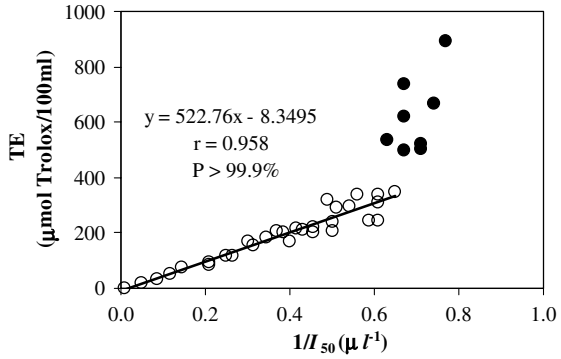

Fig. 2. Correlation between DPPH· ($1/I_{50}$) and electrochemical (TE) methods for the analysis of antioxidant power. Black dots represent samples with $1/I_{50}$ above 0.6 not falling within the linear correlation.

4. Conclusions

The research carried out allowed us to highlight the fact that the relative ripeness of the walnuts is an important parameter of the production process for nocino. Irrespective of the traditional date for gathering the nuts (24 June), in order to obtain a high content of phenolic substances – which form an important constituent of the liqueur – it is advisable to gather the nuts early rather than late. In the case of the cultivar used, the firmness, evaluated as described in this study, should be no greater than 1.25 J. An instrumental measure of the firmness is thus a useful means for optimizing the degree of ripeness. In the case of early gathering, the walnuts should ideally be left to steep in alcohol for approximately 3 months. On the other hand, temperature has little effect, as no substantial or systematic differences were noted between 20 and 40 °C production.

One important variable not investigated in this study, but deduced by comparison with data from previous work, consists in the cultivar used.

Table 5
Antioxidant activity (average ± s.d.) of infusions produced at 20 and at 40 °C

Sample	20 °C		40 °C	
	$1/I_{50}$ (μl^{-1})	TE (µmol trolox/100 ml)	$1/I_{50}$ (μl^{-1})	TE (µmol trolox/100 ml)
13/06-15 d	0.67 ± 0.01^{aB}	620 ± 45^{aC}	0.71 ± 0.01^{aB}	501 ± 50^{aB}
13/06-40 d	0.67 ± 0.01^{aC}	736 ± 34^{bB}	0.74 ± 0.04^{aB}	669 ± 86^{bB}
13/06-90 d	0.77 ± 0.01^{bC}	894 ± 54^{cC}	0.71 ± 0.01^{aB}	520 ± 36^{aB}
24/06-15 d	0.61 ± 0.08^{aAB}	340 ± 33^{aB}	0.65 ± 0.03^{aB}	349 ± 15^{bA}
24/06-40 d	0.59 ± 0.01^{aB}	n.d.	0.61 ± 0.03^{aAB}	242 ± 16^{aA}
24/06-90 d	0.63 ± 0.01^{aB}	533 ± 78^{bB}	0.67 ± 0.01^{aAB}	497 ± 49^{cB}
04/07-15 d	0.43 ± 0.01^{aA}	211 ± 15^{aA}	0.56 ± 0.01^{abA}	338 ± 28^{bA}
04/07-40 d	0.49 ± 0.02^{abA}	317 ± 36^{bA}	0.51 ± 0.02^{aA}	293 ± 23^{aA}
04/07-90 d	0.54 ± 0.02^{bA}	295 ± 17^{bA}	0.61 ± 0.03^{aA}	309 ± 18^{aA}

n.d., not determined.
[a,b,c] Averages with different letters indicate significant differences ($P < 0.05$) between samples produced from the same walnut batch.
[A,B,C] Averages with different letters indicate significant differences ($P < 0.05$) between samples produced with the same steeping length.

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