

A Reappraisal of the Differences Between Scandinavian and Spanish Barleys: Effect of β -Glucan Content and Degradation on Malt Extract Yield in the cv. *Scarlett*

J.-L. Molina-Cano,^{1,6} E. Romera,¹ R. Aikasalo,² A.M. Pérez-Vendrell,³
J. Larsen,⁴ and A. Rubió⁵

ABSTRACT

J. Inst. Brew. 108(2), 221–226, 2002

Forty one samples of the malting barley cultivar *Scarlett* were collected from both Scandinavia (15 from Finland and 10 from Denmark) and the Iberian Peninsula (15 from Spain and 1 from Portugal), during the harvest years of 1998 and 1999. These samples were subjected to grain analyses, comprising protein content, hordein fractions by high performance liquid chromatography (HPLC) and β -glucan content. The samples were micro-malted and the malts were analysed to determine different patterns in the influence of grain composition on malt extract development linked to the two contrasting environments. The most obvious difference found between the Scandinavian and Iberian barleys was the effect of the total and insoluble barley β -glucans. They were an effective barrier of malt extract in the North, but appeared to increase extract in the South. A conclusion was that the positive effect of β -glucans in the Iberian barleys was a consequence of their greater capacity to synthesise and release β -glucan hydrolases during germination.

Key words: Barley, β -glucans, malting quality.

INTRODUCTION

The environmentally induced differences in barley and malt composition observed between samples from such contrasting regions of Northern and Southern Europe as NE Spain and E Scotland, have been analysed for different characteristics and barley genotypes^{2,8-10,17,18}. These studies addressed different aspects, but had a common disadvantage in attempting to analyse genotypic differences when the environmental effects were considerably greater^{2,9,16}. In an early study by Molina-Cano and co-workers a pair of near-isogenic barley lines were used, and the data for both genotypes showed a positive effect of the B/C hordein ratio and the water-soluble fraction of

β -glucans on water uptake during steeping⁹. Additionally, the capacity of the Spanish barleys to give equivalent extracts to their Scottish counterparts, despite higher levels of protein and β -glucans, was attributed to their different hordein composition and their greater ability to produce β -glucanases during germination².

Subsequent studies focused on comparing environments, using barleys from the North and South of Europe⁸. However, when samples of the variety *Alexis* grown both in Scandinavia and the Iberian Peninsula were analysed, B-hordeins showed a strong negative effect along with barley protein content on the extract development of the Nordic (Scandinavian) samples⁸. In an attempt to clarify these apparently inconsistent results, we have studied the malting behaviour of the barley variety *Scarlett*, presently widely accepted by the brewing industry and successfully grown in many European regions.

MATERIALS AND METHODS

Barley material

Forty one samples of the malting barley variety *Scarlett* were collected in Scandinavia (25 samples) and the Iberian Peninsula (16 samples) in 1998 and 1999 (Table I). The Scandinavian samples were from Denmark (10 samples) and Finland (15 samples) and those of the Iberian Peninsula were from Spain (14 samples) and Portugal (1 sample).

The most marked difference between these two European regions is that, in the Scandinavian countries, spring barley varieties are spring-sown by definition, with a growing period from March to August. By contrast, a great deal of spring barley in the Iberian Peninsula is autumn-sown. Thus Scandinavian barleys are grown under a regime of long days and mild temperatures, while, in the Iberian Peninsula, barley experiences both short and long days in addition to both cold (winter) and hot (spring) temperatures.

Micromalting and malt analyses

Barley samples were screened over a 2.2 mm sieve and the grains >2.2 mm were collected for further use. The total barley protein content was analysed prior to micromalting, which was carried out according to the procedure previously described¹¹. Malt analyses were carried out ac-

¹ Centre UdL-IRTA, Av. Rovira Roure, 177, 25058 Lleida, Spain

² Boreal Plant Breeding, 3600 Jokioinen, Finland

³ IRTA, Centre Mas Bové, 43280 Reus, Spain

⁴ Carlsberg Research Centre, 2500 Valby-Copenhagen, Denmark

⁵ La Moravia-Damm Malting Company, Bell-lloc (Lleida), Spain

⁶ Corresponding author. E-mail: joseluis.molina@irta.es

ording to the EBC official methods³, for extract yield, total and soluble protein content, Kolbach index, apparent final attenuation and viscosity of Congress wort.

Barley hordein fractionation and β -glucan analysis

Hordein fractionation was carried out with high performance liquid chromatography (HPLC), following the method of Marchylo et al.^{4,5}. β -Glucan analysis was carried out by an enzymatic method⁷, determining both the total content and the fraction insoluble in water at 38°C.

Statistical analysis

Analysis of variance (general linear model), box-and-whisker plots and principal component analysis were carried out with Statgraphics 4.1¹⁵. Principal component analysis consists of transforming a set of variables, x_1, \dots, x_p , into a new set, y_1, \dots, y_p , so that each principal com-

ponent represents an uncorrelated linear combination of original variables. Most of the variability can thus be summarised into the first few principal components, i.e. those with the highest variances, simplifying interpretation of the original data⁶ and easing consideration of the differences between samples. The correlation matrix of the standardised values of the quality characters was used to calculate the eigenvalues of the covariance matrix and the principal components. The choice of this matrix is justified because the quality characters were measured in different units, so it was necessary to devise a uniform scale.

RESULTS AND DISCUSSION

Environmental effects on grain and malt quality parameters

The *Scarlett* barley samples derived from sites representative of the wide contrasts in barley growing area,

TABLE I. Geographical and climatic data on the sites where the *Scarlett* barley samples were collected. (FI: Finland, DK: Denmark, S: Spain, P: Portugal)

Country	Site	Year	Longitude	Latitude	Altitude (m)	Rainfall ¹	Temperature ²	Sowing time ³	Heading time ⁴	Maturity time ⁵
FI	Mietoinen	98	60°38' N	21°51' E	14	92.6	14.9	May	June	August
FI	Mietoinen	98	60°38' N	21°51' E	14	123.3	14.6	May	June	August
FI	Vihti	98	60°21' N	24°23' E	49	139.4	13.4	May	June	August
FI	Jokioinen	98	60°49' N	23°29' E	104	117.3	13.8	May	June	August
FI	Pälkäne	98	61°20' N	24°13' E	100	159.6	14.3	May	June	August
FI	Jokioinen	98	60°49' N	23°29' E	104	118.0	13.8	May	June	August
FI	Jokioinen	98	60°49' N	23°29' E	104	117.9	13.5	May	June	August
FI	Jokioinen	99	60°49' N	23°29' E	104	40.1	17.8	May	June	August
FI	Pälkäne	99	61°20' N	24°13' E	100	48.6	17.2	May	June	August
FI	Inkoo	99	60°05' N	24°00' E	23	53.7	17.2	May	June	August
FI	Ylistaro	99	62°57' N	22°30' E	26	34.5	16.7	May	June	August
FI	Jokioinen	99	60°49' N	23°29' E	104	40.1	17.8	May	June	August
FI	Pälkäne	99	61°20' N	24°13' E	100	48.6	17.2	May	June	August
FI	Mietoinen	99	60°38' N	21°51' E	14	24.2	17.3	May	June	August
FI	Jokioinen	99	60°49' N	23°29' E	14	40.1	17.8	May	June	August
DK	Sejet	99	55°50' N	10°00' E	10	55.0	17.6	March	June	August
DK	Nr. Aaby	99	55°30' N	09°50' E	15	56.0	17.5	March	June	August
DK	Roskilde	99	55°40' N	12°10' E	20	26.0	17.5	March	June	August
DK	Naestved	99	55°15' N	11°40' E	15	53.0	17.3	March	June	August
DK	Karise	99	55°20' N	12°10' E	20	53.0	17.3	March	June	August
DK	Stevns	99	55°20' N	12°20' E	20	53.0	17.3	March	June	August
DK	Mon	99	54°55' N	12°20' E	25	53.0	18.1	March	June	August
DK	Brovst	99	57°05' N	09°20' E	30	62.0	16.4	March	June	August
DK	Fjellerad	99	57°00' N	10°10' E	20	62.0	16.4	March	June	August
DK	Hadsund	99	56°45' N	10°00' E	40	49.0	16.7	March	June	August
S	Alava1	99	42°45' N	03°03' W	473	46.1	19.1	February	June	July
S	Alava2	99	42°51' N	02°38' W	515	47.3	17.3	February	June	July
S	Huesca	99	42°05' N	00°26' W	488	63.0	20.7	December	May	July
S	Zaragoza	99	41°37' N	00°42' W	195	25.1	25.3	December	May	June
S	Badajoz1	99	38°46' N	06°24' W	235	57.0	22.1	November	April	June
S	Alava3	99	42°51' N	02°39' W	545	49.5	17.5	February	June	July
S	Burgos	98	42°12' N	03°40' W	814	48.6	21.0	February	June	August
S	Valladolid	98	41°34' N	04°42' W	770	22.4	20.5	January	June	July
S	Badajoz	98	38°45' N	06°21' W	221	50.0	19.1	November	April	June
S	Albacete	98	39°05' N	01°58' W	686	26.1	19.3	December	April	June
P	Elvas	98	40°05' N	07°10' W	300	56.3	20.8	November	April	June
S	Salamanca1	99	40°07' N	05°09' W	868	29.0	19.9	November	May	June
S	Soria	99	41°51' N	02°12' W	1062	31.2	18.2	February	June	June
S	Albacete	99	39°05' N	01°58' W	686	26.4	19.5	January	May	June
S	Badajoz2	99	38°45' N	06°21' W	221	50.4	20.1	November	April	June
S	Salamanca2	99	40°05' N	05°11' W	899	32.4	19.0	November	May	July

¹Total rainfall during grain filling period.

²Mean temperature during grain filling period.

³Months when sowing occurred, as an average.

⁴Months when heading occurred, as an average.

⁵Months when maturity occurred, as an average.

both in latitude and longitude and, particularly in Spain, in altitude (Table I). Most environmental conditions of the barley culture were thus explored. The contrasting growing regimes of Northern and Southern Europe were exemplified by the differences in sowing time (spring in Scandinavia and autumn-winter in the Iberian Peninsula), heading time (summer in Scandinavia and spring in the Iberian Peninsula), and maturity (late summer in the northern region and early summer in the South). The grain development period is, therefore, more humid, with milder temperatures, in Scandinavia than in the Iberian Peninsula. However, the extent of variation within both regions is also very large.

Variation in the most relevant of these quality parameters is presented graphically in Fig. 1, where the box-and-whisker plots show the extent of the total variation within either of the two regions. This type of plot summarises a set of univariate observations and enables comparison of distributions and identification of outliers. The data is divided into four areas of equal frequency. The box encloses the middle 50 % of the data. The median is drawn as a vertical line inside the box and the mean is a cross, also

inside the box. Two horizontal lines, called whiskers, extend from each end of the box, the left whisker extends from the first quartile to the smallest data point within 1.5 interquartile ranges of it, while the right one, extends from the third quartile to the largest data point within 1.5 interquartile ranges of it.

Some of the distributions seem to be skewed towards the lower values (barley protein and D hordein at both regions) or to the higher (total and insoluble barley β -glucans in the South).

The arithmetic means of the grain and malt quality parameters are listed by regions (Scandinavia and Iberian Peninsula) in Table II, together with their level of significance after analyses of variance on the raw data. Equivalent mean levels were attained at both regions for most of the barley and malt quality parameters. However, the Scandinavian samples of *Scarlett*, when compared with their Iberian Peninsula counterparts, had slightly higher malt extract yield (82.46 vs. 81.62%; $p < 0.05$). They also showed higher total β -glucan levels in barley (4.48 vs. 4.12%; $p < 0.05$) and malt (1.53 vs. 1.06%; $p < 0.05$), but the relative proportion of total barley β -glucans degraded dur-

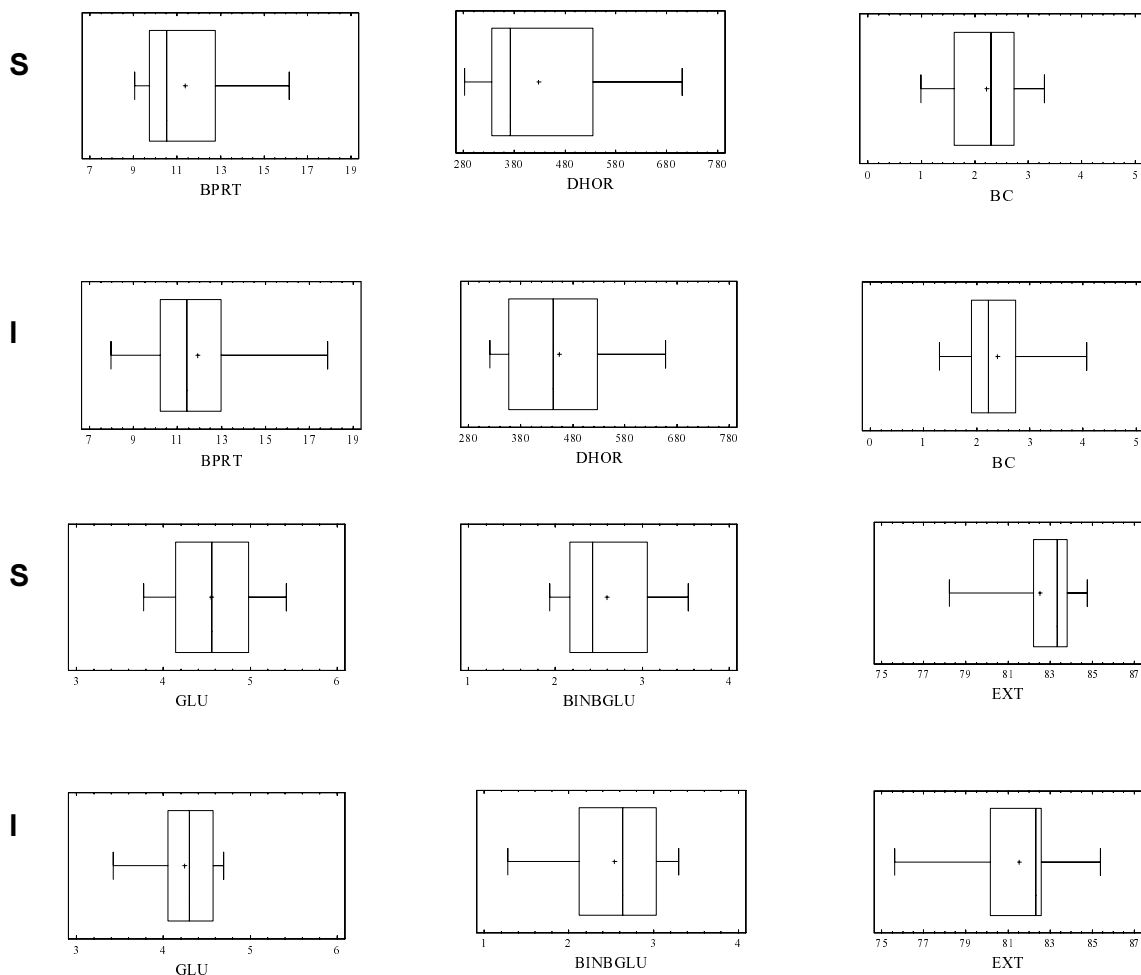


FIG. 1. Box-and-whisker plots of several quality parameters in *Scarlett* barley samples from Scandinavia and the Iberian Peninsula. Details of the plotting technique are given in the text. S: Scandinavia, I: Iberian Peninsula, BPRT: barley total protein content (%), DHOR: barley D-hordein content (arbitrary units), BC: ratio of barley B to C hordein. BTBGLU: barley TOTAL β -glucans (%), BINBGLU: barley insoluble β -glucans (%), EXT: malt extract yield (%).

ing malting, was significantly smaller (66.92 vs. 75.79%; $p < 0.05$). The more incomplete digestion of β -glucans led to a higher viscosity in the Scandinavian worts (1.60 vs. 1.51 mPa.s; $p < 0.05$). These results suggest a higher β -glucanase activity in the Iberian barleys, as observed in previous work^{2,18} and consequently, a more thorough cell wall modification, although some other factors, such as cell wall thickness, cross-linking between β -glucans and protein, mealiness/steeliness of the grain, etc. may exist among the large amount of samples examined.

The protein degradation of the Scandinavian barleys was more limited than in the southern ones, with lower wort soluble protein (4.94 vs. 5.30%; $p < 0.05$) and Kolbach index (44.71 vs. 45.57; $p < 0.05$). Despite the higher proportion of proteinaceous material in the southern worts, Scandinavian samples also showed lower fermentabilities, as apparent final attenuation was also smaller (82.51 vs. 83.73%; $p < 0.05$). These data suggest differences in wort carbohydrate profiles that may result from limited amyolytic activity in the *Scarlett* samples from the North of Europe. Similar results have been observed when comparing Spanish vs. Scottish barleys for both α -amylase activity^{12,18} and β -amylase activity¹⁷. Additionally, as β -glucanase would still be active in the early stages of mashing, the fermentable carbohydrates may comprise glucose in the products of cell wall breakdown.

These results suggest that similar extract levels are obtained by different mechanisms in the two groups of barleys. The environment may thus be hypothesised to induce differences in the metabolic pathways that affect germination, these differences being of quantitative type, i.e. linked to the rate of germination and, therefore, to the quantity of enzyme produced at the end of it. Ultimately, all these processes might be a consequence of the hormonal differences existing between Nordic and southern barleys, as shown by Romagosa et al.¹⁴ who found higher abscisic acid (ABA) levels in Scottish compared to Spanish barleys. ABA is known to influence the dormancy/

germination system by repressing germination (reviewed in¹).

Malt extract development in Scandinavian vs. Iberian barleys

The existing differences between these two groups of barleys were explored using principal component analysis. The advantage of this statistical technique is that it allows interpretation of complex phenomena with the aid of a two-dimensional graph, as described above.

Several consecutive rounds of calculation were carried out, eliminating each time any variable that did not show any effect on extract yield, or correlated variables whose effect was of the same size. This aimed to maximise the percentage of variance explained by the first two principal components. Among the correlated variables, all the hordein fractions were strongly and significantly correlated ($p < 0.05$) with extract yield negatively in both regions. In the final model, therefore, only D-hordein and B/C ratio have been retained as representatives. The same applies to barley soluble β -glucans (correlated with barley total β -glucans) and wort soluble protein (correlated with total barley protein).

In Fig. 2, the principal component analysis graphs of the Scandinavian (top) and Iberian (bottom) barleys are presented. With the Nordic samples, the two first axes accounted for 85% of the total variation, and 66% in the Iberian. Both graphs therefore contain enough information to explore the overall trends in both sets of quality data.

In both cases, higher malt extract yields were associated with the negative direction of Component 1, while barley protein increased in the opposite direction. Malt extract increased with the increases in Kolbach index, apparent final attenuation, B/C hordein ratio and β -glucan degradation. Conversely, it decreased when barley protein, D-hordein, malt total β -glucan and wort viscosity increased.

There is, however, a very clear difference between

TABLE II. Means of a set of barley and malt quality parameters in samples of the cv. *Scarlett* from Scandinavia and the Iberian Peninsula, harvested in 1998 and 1999.

Quality parameter	Scandinavia ^{1,2}	Iberian Peninsula ^{1,3}
Barley protein (N x 6.25, %)	11.60a	11.91a
Barley B hordein (arbitrary units)	4554a	4800a
Barley C hordein (arbitrary units)	2558a	2315a
Barley D hordein (arbitrary units)	441a	454a
Barley total hordein (arbitrary units)	7553a	7568a
Barley B/C hordein ratio	2.15a	2.39a
Barley B/D hordein ratio	10.45a	10.49a
Barley C/D hordein ratio	5.37a	4.85a
Barley total β -glucans (%)	4.48a	4.12b
Barley soluble β -glucans (%)	1.85a	1.66a
Barley insoluble β -glucans (%)	2.72a	2.63a
Malt extract yield (%)	82.46a	81.62b
Malt total protein (%)	10.90a	11.08a
Wort soluble protein (%)	4.94a	5.30b
Malt Kolbach index	44.71a	45.57b
Wort viscosity (mPa.s)	1.60a	1.51b
Wort apparent final attenuation (%)	82.51a	83.73b
Malt total β -glucans (%)	1.53a	1.06b
β -glucans degradation (%) ⁴	66.92a	75.79b

¹ Means, in bold type, followed by a different letter are significantly different at least at $p < 0.05$ after analysis of variance.

² Scandinavia, n=25 samples.

³ Iberian Peninsula, n=16 samples.

⁴ Percent of total barley β -glucans – total malt β -glucans.

Scandinavian and Iberian barleys in Fig. 2, i.e. the opposite way in which total barley β -glucan and its insoluble fraction affect malt extract. They are positively associated with increases in extract in the Iberian samples, but cause decreases in Scandinavian barleys. Additionally, there is a quantitative difference between these barleys with regard to wort viscosity ($p < 0.05$). It has a greater negative effect on malt extract in the Scandinavian than in the Iberian, indicating less complete β -glucan hydrolysis.

These results may be explained by the difference of enzyme levels between these groups of barleys. In the South, the higher total and, particularly, insoluble barley β -glucan content may have contributed positively to increases in extract because they are degraded to a large extent and add fermentable material to the wort. This could result from the higher enzymatic capacity of these barleys, as shown by Ellis et al.². Barley β -glucan content would not, therefore, be a positive trait unless it was linked to a high β -glucanase activity. The very different environmental conditions between the barley growing regimes in Northern and Southern Europe may alter the hormonal balance which, ultimately, conditions the capacity of the aleurone

layer to synthesise and release the hydrolytic enzymes that will degrade the endosperm reserves, leading to a higher quantity of solutes in the wort.

The results presented here do not totally agree with those previously published on models of malt extract development in Scandinavia and the Iberian Peninsula⁸. Previously there was no recorded effect of β -glucans that was responsible for the differences between these two groups of barleys. Conversely, only total barley protein and B-hordeins appeared to have a role in explaining the differences in extract development. These differences were of quantitative (size) but not qualitative (sign) nature, because both negatively influenced extract, albeit to a different extent, in both regions. The effects of protein have also been shown in the present research.

The reasons for the discrepancies between these two studies, regarding the effect of β -glucans, may be complex. However the use of different cultivars (*Alexis* vs. *Scarlett*) might influence the characteristics analysed in a different way, as shown in studies of Spanish vs. Scottish barleys^{2,8-10,17,18}. In these, the cultivar effect was always significant, albeit of smaller size than the effect of environment. The same result has been obtained by many other workers studying the genetic and environmental effects on malting quality characters (reviewed in¹³).

The final conclusion of the present experiment is that, depending on cultivar, the environment may modify the quantity of β -glucans synthesised and the differential development of enzymes able to degrade them into fermentable sugars and thus extract. The products of cell wall modification could thus enhance the final extract. These results should be validated by using commercial lots of barley and malt, since at industrial level only small quality differences induced by environment into barley and malt batches can be tolerated.

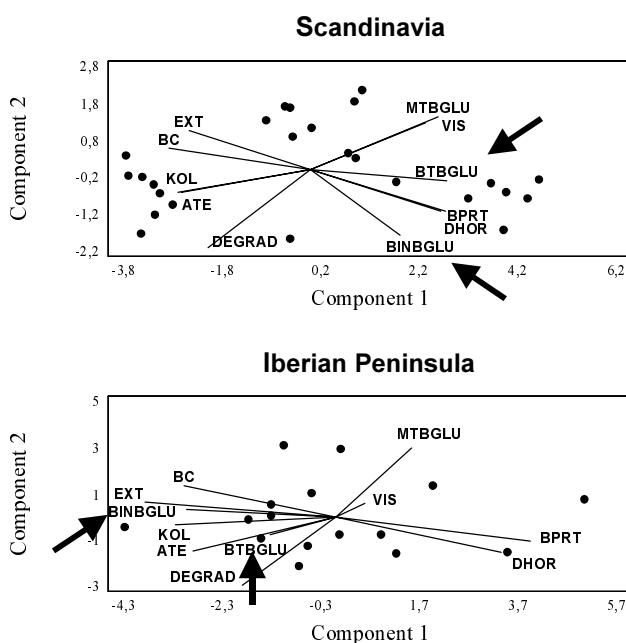


FIG. 2. Principal component analysis graphs of several quality parameters in *Scarlett* barley samples from Scandinavia (top) and the Iberian Peninsula (bottom). The points represent the different *Scarlett* samples. EXT: malt extract yield (%), BINBGLU: barley insoluble β -glucans (%), KOL: Kolbach index (%), ATE: apparent final attenuation (%), BTBGLU: barley total β -glucans (%), DEGRAD: β -glucan degradation (BTBGLU-MTBGLU (%), DHOR: barley D-hordein content (arbitrary units), BPRT: total barley protein (%), VIS: wort viscosity (mPa.s), MTBGLU: malt total β -glucans (%), BC: ratio of barley B to C hordein. The lines are vectors representing the quality characters studied. The length of the projection of each of them on each principal component axis measures the weight of its influence on that axis, whereas the angle between each two vectors is inversely proportional to the correlation existing between them.

ACKNOWLEDGEMENTS

We thank Comisión Interministerial de Ciencia y Tecnología (CICYT) for partially funding this research. We want also to thank Dr. J.S. Swanston (SCRI, Dundee, UK) for reading the manuscript critically.

REFERENCES

1. Corbineau, F. and Côme, D., *Bios*, 1996, **261**, 113.
2. Ellis, R.P., Swanston, J.S., Rubió, A., Pérez-Vendrell, A.M., Romagosa, I. and Molina-Cano, J.-L., *Journal of Cereal Science*, 1997, **26**, 75.
3. European Brewery Convention. Analytica EBC, 4th ed. Brauerei- und Getranke- Rundschau, Zurich, Switzerland, 1987.
4. Marchylo, B.A., Hatcher, D.W., Kruger, J.E. and Kirkland, J.J., *Cereal Chemistry*, 1992, **69**, 371.
5. Marchylo, B.A. and Kruger, J.E., *Cereal Chemistry*, 1984, **61**, 295.
6. Marriott, F.H.C., *The Interpretation of Multiple Observations*, Academic Press: New York, 1974, pp. 18-25.
7. McCleary, B.V. and Glennie-Holmes, M., *Journal of the Institute of Brewing*, 1985, **91**, 285.
8. Molina-Cano, J.-L., Polo, J.P., Sopena, A., Voltas, J., Pérez-Vendrell, A.M. and Romagosa, I., *Journal of the Institute of Brewing*, 2000, **106**, 117.
9. Molina-Cano, J.-L., Ramo, T., Ellis, R.P., Swanston, J.S., Bain, H., Uribe-Echeverría, T. and Pérez-Vendrell, A.M., *Journal of the Institute of Brewing*, 1995, **101**, 79.

10. Molina-Cano, J.-L., Rubió, A., Igartua, E., Gracia, P. and Montoya, J.L., *Journal of the Institute of Brewing*, 2000, **106**, 111.
11. Molina-Cano, J.-L., Rubió, A., Royo, C., Ramo, T., Pérez-Vendrell, A. and Palmer, G.H., Proceedings of the European Brewery Convention, Congress, Lisbon, IRL Press: Oxford, 1992, pp. 69-76.
12. Molina-Cano, J.-L., Sopena, A., Swanston, J.S., Casas, A.M., Moralejo, M.A., Ubieto, A., Lara, I., Pérez-Vendrell, A.M. and Romagosa, I., *Theoretical and Applied Genetics*, 1999, **98**, 347.
13. Molina-Cano, J.-L., Swanston, J.S. and Ullrich, S.E., 8th International Barley Genetics Symposium, Adelaide, Australia. S. Logue, Ed., University of Adelaide, 2000, Vol. 1, pp. 127-134.
14. Romagosa, I., Prada, D., Moralejo, M.A., Sopena, A., Muñoz, P., Casas, A.M., Swanston, J.S. and Molina-Cano, J.-L., *Journal of Experimental Botany*, 2001, **52**, 1499.
15. Statistical Graphics Corporation. Statgraphics Plus 4.1, Manugistics, Rockville, Maryland 20852, USA, 1999.
16. Swanston, J.S., Ellis, R.P., Pérez-Vendrell, A., Voltas, J. and Molina-Cano, J.-L., *Cereal Chemistry*, 1997, **74**, 456.
17. Swanston, J.S. and Molina-Cano, J.-L., *Journal of Cereal Science*, 2001, **33**, 155.
18. Swanston, J.S., Ellis, R.P., Rubió, A., Pérez-Vendrell, A. and Molina-Cano, J.-L., *Journal of the Institute of Brewing*, 1995, **101**, 261.

(Manuscript accepted for publication April 2002)