JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effects of the Combination of Hydrophobic Polypeptides, Iso-α Acids, and Malto-oligosaccharides on Beer Foam Stability

ISABEL M. P. L. V. O. FERREIRA,*[,†] KÁTIA JORGE,[‡] LUCIANA C. NOGUEIRA,[‡] FILIPE SILVA,[†] AND LUIZ C. TRUGO[‡,§]

REQUIMTE—Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Portugal, and Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

The influence of hydrophobic polypeptides concentrated in beer foam, together with the composition of iso-α acids and the content of malto-oligosaccharides in beer on foam stability, has been investigated. The objective was to find out whether a shortage of one of these positive contributors to foam stability could be compensated for by an increased presence of another or whether optimum levels of each contributor is necessary. For that purpose, an image analysis method to evaluate beer foam quality was developed. The foam collapse time was the parameter chosen to group beers according to their foam stability. Profiles of hydrophobic polypeptides that concentrate in beer foam, iso-α acids, and malto-oligosaccharides of 14 beer brands were acquired by high-performance liquid chromatography. Principal component analysis (PCA) was performed to show the relationship between beer brands and its composition. Beers that contained propylene glycol alginate as a foam enhancer showed high foam stability except for one beer, which had a low content of hydrophobic polypeptides, thereby highlighting the requirement of threshold levels of hydrophobic polypeptides to obtain stable foam. The data of samples that were devoid of a foam additive were subjected to a discriminant statistical analysis. Foam stability declined in proportion to decreases in hydrophobic polypeptides and to a lesser extent to decreases in iso-α-acid contents. Apparently, the content of malto-oligosaccharides were found to have no major influence on foam stability. The model of discriminate analysis was found to explain 100% of the variance in data with 85.2% success in classifying all samples according to the model, suggesting that foam stability is mainly governed by the beer constituents evaluated in this study.

KEYWORDS: **Beer; foam stability; hydrophobic polypeptides; iso-α acids; malto-oligosaccharides**

INTRODUCTION

Various parameters define beer foam quality including foam stability or head retention, foam texture, and foam adhesion or foam cling (*1*). Foam texture is related to the bubble size. Fine-textured foam consists of small bubbles, whereas coarse foam contains larger bubbles. Foam adhesion is considered to be related to the formation of foam lace to the glass. Methods available for measuring these features are usually based on measurements of the weight or the volume of the liquid collapsed from the foam (*2*−*7*). However, liquid drainage is only one of the factors related to foam quality. Consequently, values measured by conventional drainage methods may give rise to a misleading evaluation of foam quality. According to Evans and Sheehan (*1*), the best method to access foam quality must combine a digital camera and software for image analysis, which uses algorithms to reproduce consumer's evaluation. Several authors have used visual systems of foam evaluation [Constant (*5*), Hallgren et al. (*8*), Jorge et al (*9*), Skandes et al. (*10*), Hedarty et al. (*11*), and Yasui et al. (*12*)].

The structure of foam is complex, with a network of hexagonal bubbles, the walls of which comprise surface-active components. It has long been a matter of major interest to establish the chemical nature of those materials in beer that contribute to the stability of the foam and mutual interactions that give rise to a desirable foaming quality (*13*). Fractionation of beer constituents based on size and hydrophobicity has been used to identify key surface-active compounds. A pivotal role in stabilizing beer foam is attributed to barley proteins or polypeptides that interact with bittering agents derived from hops (iso-α acids) to form a matrix (*13*).

There are two schools of thought concerning the nature of the foaming proteins in beer. On one hand, it is claimed that specific proteins including protein Z (*14*−*16*) and lipid transfer protein (LTP1) are essential (*17*−*19*); on the other hand, it is put forward that a diversity of amphiphilic polypeptides may serve to stabilize foam, whereby the foam activity parallels the overall hydrophobic nature (*20*, *21*). Iso-α acids, the bittering

* To whom correspondence should be addressed. Telephone: +351-222078929. Fax: +351-222003977. E-mail: isabel.ferreira@ff.up.pt.
[†] Universidade do Porto.
[‡] Universidade Federal do Rio de Janeiro.
[§] Deceased.

10.1021/jf047796w CCC: $30.25 © 2005 American Chemical Society
Published on Web 05/17/2005

Table 1. Characterization of Beer Brands

beer brand	alcohol (percent volume)	ingredients on the label
1	4.8	water, malt, rice, hop
2	5.3	water, malt, wheat, hop; re-fermented in the bottle
3	5.1	water, malt, unmalted cereals, hop, antioxidant (E224)
4	5.4	water, malt, rice, maize, hop
5	8	water, malt, starch from wheat, sugar, hop; re-fermented in the bottle
6	7.2	water, malt, unmalted cereals, sugars, hop, antioxidant (E224)
7	5.4	100% malt
8	5.6	water, malt, unmalted cereals, glucose syrup, hop, antioxidant (E224)
9	5.4	water, malt, rice, hop, stabilizer (E405), colorant (E150)
10	alcohol free	water, malt, rice, maize, hop, stabilizer (E405)
11	alcohol free	water, malt, unmalted cereals, hop, stabilizer (E405)
12	7.2	water, malt, rice, maize, hop, stabilizer (E405)
13	4.8	water, malt, rice, maize, hop, stabilizer (E405)
14	4.6	water, malt, hop, rice/maize, stabilizer (E405)

components that are formed during wort boiling from precursors (α acids) present in hops (*Humulus lupulus*), contribute to foam stability. It is generally accepted that the fundamental basis for beer foam stability is the interaction between iso-α acids and barley polypeptides (*13*−*24*), which increases the viscosity of the liquid regions in the foam, reduces drainage, and improves foam stability. Simpson and Hughes (*13*) proposed a model in which polypeptides present in the inner and outer faces of the film surrounding the bubbles are intimately connected via complexation with iso-α acids, which in turn form very stable complexes with bridging metal cations. Two types of interactions between iso-α acids and barley polypeptides are evident. First, the amino groups of the polypeptides and the carbonyl groups of the iso-α acids interact by ion−dipole binding. Second, further stabilization of the foam matrix results from hydrophobic binding between the prenyl side chains of the iso-α acids and hydrophobic amino acid residues in the polypeptides (*13*).

The need to attract consumers by presentation of beers with a stable foam head led to the use of so-called advanced hop products (reduced iso-α acids) in beer brewing, including tetrahydroiso-α acids and hexahydroiso-α acids. These compounds enhance foam stability to a greater extent than do iso-α acids as a result of the increased hydrophobicity, thereby enhancing their ability to participate in hydrophobic interactions (*24*, *25*). Oligosaccharides and polysaccharides are named by some authors as possible foam-enhancing compounds by virtue of their propensity to increase localized viscosity, which leads to slowing down liquid drainage and, consequently, to improving foam stability (*1*, *3*). It should also be added that propylene glycol alginate (PGA) can be used as a foam additive (*1*). This is a heterogeneous substance formed by the partial esterification of alginic acid with propylene oxide. The degree of esterification is important in determining the efficacy of foam stabilization and colloidal stability of the final beer and is typically 80−90 (*26*). Thus, PGA contributes to maintain a creamier foam with a uniform and natural appearance. The mechanism by which PGA stabilizes foam is unclear, although it may be due to electrostatic interactions that take place between the carboxyl groups of PGA and the amino groups of peptides in foam bubbles and form a coat around the bubbles, altering the surface tension and reducing the rate of bubble drainage. Typical levels of application are 50 mg L^{-1}.

While the contributions of varying beer constituents have been investigated repeatedly, it is still unclear whether a lack of one of these positive contributors can be compensated for by an increased concentration of another contributor or whether optimum levels of each component is necessary. We have mainly focused on the study of foam stability and investigated the influence of hydrophobic polypeptides concentrated in beer foam, in connection with the contents of iso-α acids and malto-oligosaccharides. Because most methods for the assessment of foam stability appear to provide a misleading evaluation of foam quality, one of the goals of this work was to adapt a method based on image analyses and to compare the results with those following the ASBC procedure (σ-value method) (*27*). It was a further aim to compare the compositions of hydrophobic polypeptides in the beer foam of iso-α acids and of malto-oligosaccharides by high-performance liquid chromatography (HPLC) using diode array, UV, and light-scattering detectors. Finally, multivariate statistical analyses, which included principal component analysis and discriminant analysis, were performed to correlate the composition of beer constituents with foam stability.

MATERIALS AND METHODS

Samples. A total of 14 commercial beer brands were acquired according to their composition (**Table 1**). Beers were bought at local stores, kept at room temperature, and freshly opened prior to analysis.

Reagents and Standards. All reagents used were of analytical-grade purity. Solvents for HPLC were filtered through 0.22 μm NL 17 filters (Teknokroma, Madrid, Spain) and degassed under vacuum for at least 15 min before use. Iso-α acid, dihydroiso-α acid, and tetrahydroiso-α acid standards were a generous gift from Brew Tech (Rio de Janeiro, Brazil). The standards were dissolved separately in methanol (LiCrosolv, Merck, Darmstadt, Germany) to a concentration of 1 mg/mL. These stock solutions were stored in the freezer and used to prepare standard solutions.

Maltotriose, maltotetraose, fructose, phosphoric acid, tetrabutylammonium hydroxide, and trifluoracetic acid were supplied by Sigma Chemicals (St. Louis, MO); glucose was from Merck (Darmstadt, Germany); and maltose was purchased from Fluka (Seelze, Germany). Oligosaccharides standard solutions were prepared in a mixture of 50% water and 50% acetonitrile (v/v) (Merck, Darmstadt, Germany).

Measurement of Beer Foam. *Image Analysis.* A visual system for evaluation of foam analysis was developed. Beer samples were held at 6 °C, and analyses were performed at ambient temperature (ca. 20 °C). The foam cylinder was the same of that used by Ross and Clark (modified Carlsberg method) (*27*); an amount of 800 mL of foam was generated to reach the upper cylinder's mark. The distance between the beer bottle/can and the cylinder was 10 cm. The beer was photographed every 30 s for a total of 300 s. The time ranging between starting generation of foam and the first photograph was keep constant (10 s). A digital camera, Kodak DC120 (Rochester, NY), and a software Kodak Digital Science 1 D were used (Rochester, NY).

The foam cylinder and the digital camera were facing each other (50 cm distance), and a light source was mounted above the foam cylinder (50 cm distance). A specific rectangular area was defined at the foam cylinder glass surface (see **Figure 1**). Kodak Scientific

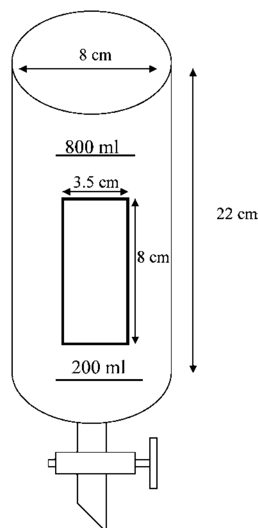

Figure 1. Representation of the foam cylinder, with dimensions marked. A specific rectangular area was defined at the foam cylinder glass surface (8 × 3.5 cm).

Imaging Systems offer innovative digital imaging and image analysis systems for electrophoresis gels, blots, plates, and others, such as finding bubbles on an image. The algorithm determines the bubble location and accurately calculates the spacing of the bubbles on the image. Once the bubbles on the image are marked and labeled, the average number of bubbles of each image can be determined in the command "Find bands"; this parameter was named visual foam index (VFI). Beer foam samples with medium- and large-size bubbles give high VFI values (high number of bands), because their bubbles are easily detectable. Inversely, small-size bubbles will not be efficiently distinguished by the software; groups of small bubbles will be detected as a single bubble and will give low VFI values.

The foam collapse time (FCT) measures the time between the first photograph and the last one until the foam fills the defined visual field for image analyses.

σ-Value Method. Beer foam analysis was also performed according to the ASBC procedure (σ-value method) (*27*). Foam is generated by pouring beer into a special foam funnel. Beer collecting in the funnel is drained away at a rate such that only foam is present in the funnel 90 s after the end of pouring. At a time period of 225−230 s after the first drainage period, the volume of beer drained from the foam and the volume of beer in the foam (c) are measured.

$$\Sigma = t/2.303 \log(b + c)/c$$

Foam is expressed as Σ values in seconds. Σ values of 105, 95−105, and <95 s describe good, average, and poor foams, respectively.

Beer Composition. Three HPLC methods were used to determine the levels of hydrophobic polypeptides in beer and beer foam as well as iso-α acids and malto-oligosaccharides in beer. All measurements were done in triplicate. Separation of hydrophobic polypeptides was performed by a reversed-phase HPLC (RP-HPLC) method optimization for this study, described later. Iso-α acids were determined by a HPLC method previously developed by Jorge and Trugo (*28*), and malto-oligosaccharides were analyzed using a HPLC method with light-scattering detection previously validated by our research group (*29*).

RP-HPLC of Hydrophobic Polypeptides. RP-HPLC separation of hydrophobic peptides that concentrate in the foam were obtained as follows. Beer (200 mL) was sparged with air through a HPLC inlet tube assembly (Ref E45275, Gilson, France) until 1 L of foam had been formed in a 2 L Erlenmeyer flask at 20 °C. The foam was allowed to drain for 15 min before the beer liquid was removed. The foam was then allowed to collapse and finally collected and filtered before HPLC analysis. The chromatographic analysis was carried out using an analytical HPLC unit (Jasco, Tokyo, Japan) equipped with a quaternary pump type PU 1580, a type 7125 Rheodyne Injector (Perkin-Elmer, Boston, MA) with a 100 μL loop, a Jasco multiwavelength diode array detector MD-910, and Borwin PDA Controller Software. The column was a Chrompack P 300 RP (polystyrenedivinylbenzene copolymer) (8 μm, 300 μm, 150 × 4.6 mm i.d., Chrompack, Middelburg, The Netherlands). A column heater type 7981, Jones Chromatography, Ltd. (South Wales, U.K.), was used, and the temperature was 45°C. Gradient elution was carried out with a mixture of solvent A [0.1% trifluoroacetic acid (TFA) in water] and solvent B [0.1% TFA in 80% aqueous acetonitrile, (v/v)]. A linear gradient with 60% B in A to 100% B over 30 min was applied, returning to the initial conditions within 5 min. The analysis was monitored at 280 nm.

Table 2. Results of the Beer Foam Analyses (σ, *T*, *b*, and *c* Values)

	beer brands	VFI	FCT	*c*	σ
samples without PGA	1	32/33	270	7.7/8.0	95/98
	2	49/50	210	8.2/8.0	91/92
	3	49/48	210	6.9/7.1	103/104
	4	52/53	180	4.2/4.0	94/93
	5	14/12	120	13.0/13.2	110/112
	6	46/48	150	10.8/10.5	123/123
	7	58/56	210	5.6/5.5	92/93
	8	57/58	210	5.8/5.4	94/93
samples with PGA	9	29/29	240	9.3/9.0	107/108
	10	47/48	240	7.5/7.8	107/110
	11	31/32	240	6.9/6.5	102/101
	12	33/34	240	14.9/15.0	120/121
	13	35/34	240	9.1/9.5	106/107
	14	27/28	180	5.6/6.0	105/107

HPLC of Iso-α Acids. The HPLC procedure was based on the method described by Jorge and Trugo (*28*). Ion-pair chromatography was applied using an isocratic HPLC system (Gilson Medical Electronics, France), which consisted of a C18 column (5 μm, 250 × 4 mm i.d.), two pumps type 302 and 305, a type 7125 Rheodyne Injector (Perkin-Elmer, Boston, MA) with a 20 μL loop, a Gilson 118 UV/vis detector, and Gilson 712 software. Elution at a flow rate of 0.5 mL/min was performed at ambient temperature.

HPLC of Malto-oligosaccharides. The chromatographic analysis was carried out using an analytical HPLC unit (Jasco, Tokyo, Japan), equipped with a low-pressure quaternary pump (PU, 1580), an evaporative light-scattering detector (LSD, Sedex 75, France), a type 7125 Rheodyne Injector (Perkin-Elmer, Boston, MA) with a 10 μL loop, and Borwin Controller Software (JMBS Developments, Le Fontanil, France). A Spherisorb NH_2 (5 μm, 250 × 4.6 mm i.d., Waters Corp., Milford, MA) was used for separation. Gradient elution was carried out with a mixture of solvent A (acetonitrile) and solvent B (water), increasing the proportion of solvent B from 19 to 25% over 40 min: 0−19 min, 19% B; 20−40 min, 25% B. The flow-rate was 1 mL/min. The temperature of the heated drift tube was 45 °C; the gas pressure was 3.0 bar; and the gain was set at 5 (*29*).

Statistical Analysis. Statistical analyses were performed with SPSS for Windows version 11.5 (SPSS, Chicago, IL). Principal component analysis was performed with data collected from the contents of hydrophobic polypeptides, iso-α acids, and malto-oligosaccharides to find similarities and differences of the 14 beers studied. Discriminant analysis was carried out with quantitative data of beers that did not contain foam additives, using foam stability as the grouping variable and total contents of polypeptides, iso-α acids, and malto-oligosaccharides as independent variables.

RESULTS AND DISCUSSION

Measurement of Beer Foam. Results from foam image analyses are presented in **Table 2**. FCT is a measure of foam stability, whereas VFI evaluates foam texture that is related to the bubble size. No correlation was found between FCT and mean VFI values.

Beers were divided into those without foam additives (numbered from 1 to 8) and those containing PGA (numbered from 9 to 14, **Table 2**). The first series represent beers with

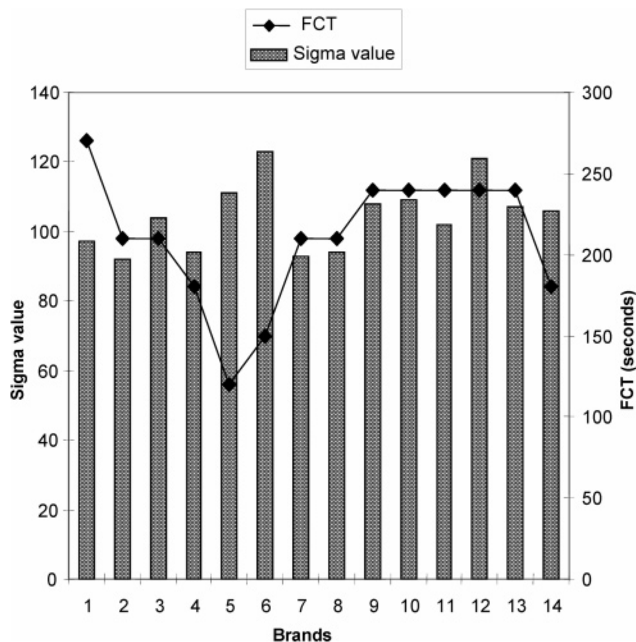

Figure 2. Comparison between FCT and σ values for 14 beer brands.

FCT values (between 120 and 270 s) and mean VFI values (between 12 and 58). The second series represent beers with comparable foam stabilities with FCT values of 210 s and mean VFI values ranging between 30 and 45, except for sample 14, which had a lower foam stability with a VFI value of 28 and a FCT value of 180 s. As expected, addition of foam-active PGA contributed to an enhancement of the foam stability.

Table 2 also presents σ and c values. Beers without PGA gave different σ values ranging from 92 to 123, whereas beers with PGA had higher σ values ranging from 102 to 121. No correlation was found between foam stability measured by FCT and σ values (**Table 2**). This is not surprising in view of the fact that different foam parameters were evaluated. FCT measures foam stability by measuring the time between the first photograph until the time of the last photograph, where foam fills the defined visual field for image analysis, while the σ value measures foam drainage together with foam adhesion.

The foam characteristics of brands 5 and 6 clearly explain the differences between these two methods (**Figure 2**). After 120 s, the image could not be analyzed by the ID software, because the foam did not totally fill the defined visual field for analysis. In view of the foam instability, the lowest σ value should have resulted for this beer. However, its c value, used to calculate the σ value, is the highest after sample 12; hence, a higher σ value is obtained.

From our experiments, the FCT value seems to be the parameter of choice to evaluate foam stability; in addition, it can be related to consumer visual impression of foam stability, as confirmed by Yasui et al. (*12*). These authors also concluded that conventional values do not have a close statistical significance to the visual foam stability. According to the FCT values obtained, beers were divided in three groups. Group 1, with FCT values lower than 120 s, included beers with very poor foam stability. Group 2, with FCT values between 150 and 180 s, presented poor foam stability, and group 3, with FCT values equal to or higher than 210 s, had good foam stability.

Beer Composition. *Hydrophobic Polypeptides.* Hydrophobic polypeptides were analyzed by HPLC with diode array detection. The hydrophobic polypeptides concentrated in the beer foam, as observed during HPLC analysis of beer samples and their foams (results not shown). Similar qualitative profiles were obtained for all beers foams; however, quantitative differences were noted in some brands. The total peak areas of hydrophobic polypeptides were measured, and results were expressed as the ratio between arbitrary units measured by the software and the lowest value obtained (**Table 3**).

Table 3. Composition of Hydrophobic Polypeptides, Iso-α Acids, and Malto-oligosaccharides ($n = 3$)

beer brand	hydrophobic polypeptides (ratio between arbitrary area units of polypeptides peaks and the lowest value)	iso-α acids (mg/L)	maltotriose and maltotetraose (g/L)
1	2.3	59	0.75
2	1.6	38	9.85
3	2.8	62	5.75
4	1.0	31	3.15
5	2.4	27	4.88
6	1.2	31	10.49
7	2.1	57	11.18
8	1.5	42	6.71
9	2.0	54	9.75
10	2.8	50	5.12
11	1.6	41	9.10
12	2.6	53	8.91
13	1.9	43	5.05
14	1.0	30	9.03

Iso-α Acids. During addition of hops to boiling wort, iso-α acids (isohumulones) are formed by isomerization of α acids (humulones) (*26*). Six major iso-α acids, *cis*-isohumulone and *trans*-isohumulone, *cis*-isocohumulone and *trans*-isocohumulone, and *cis*-isoadhumulone and *trans*-isoadhumulone were present in the beers resulting from the conversion of the three major α acids, humulone, cohumulone, and adhumulone, respectively. Their concentrations varied from 28 to 72 ppm (**Table 3**). Two beers contained tetrahydroiso-α acids, brands 12 and 13. Analyses were performed in beer samples and respective foams; however, no significant differences were noted in the data from beer end foam. Thus, only results from beer are presented in **Table 3**.

Malto-oligosaccharides. Chromatographic separation of maltose, maltotriose, and maltotetraose was performed. Peak identification was performed by comparison of the retention times of the standards. The most significant differences in the chromatographic profiles of the carbohydrates were observed for beers with and without alcohol. Only the contents of maltotriose and maltotetraose were used for further statistical analysis, because these oligosaccharides can influence beer viscosity (*27*). The contents of maltotriose and maltotetraose were between 0.22 and 2.5 g/L and 4.7 and 9.6 g/L (**Table 3**). Analyses were performed in beer samples and respective foams; no significant differences were noted. Only results from beer are presented in **Table 3**.

Principal Component Analysis. A biplot showing the relationship between beer brands and the levels of hydrophobic polypeptides, iso-α acids, and malto-oligosaccharides is presented in **Figure 3**. Beers with a high content of hydrophobic polypeptides and iso-α acids on one hand and brands presenting low contents of hydrophobic polypeptides and iso-α acids (**Table 3**) on the other hand clustered together. Only brand 5 had a high polypeptide content and low contents of iso-α acids and malto-oligosaccharides (**Table 3**).

Effects of the Combination of Hydrophobic Polypeptides, Iso-α Acids, and Malto-oligosaccharides on Foam Stability. Beer brands that contained PGA led to a high foam stability; however, the compositions of hydrophobic polypeptides, iso-α acids, and malto-oligosaccharides were different. Brand 14

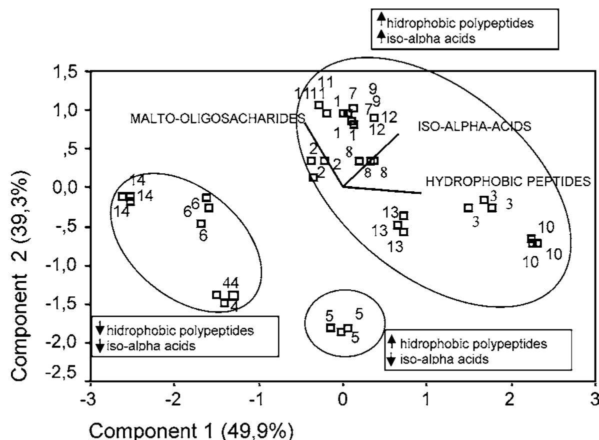

Figure 3. Principal component analysis of analytical data. Biplot showing the relationship between beer brands and levels of hydrophobic polypeptides, iso-α acids, and malto-oligosaccharides ($n = 3$).

Table 4. Standardized Coefficients of the Two Canonical Discriminant Functions (Function Values Near ± Are Significant)

	function	
	1	2
hydrophobic polypeptides	–0.996	0.928
iso-α acids	1.317	0.093
malto-oligosaccharides	0.291	0.046

presented a low foam stability because of its low content of hydrophobic polypeptides and iso-α acids (**Table 3**). Brand 9 had a high content of hydrophobic polypeptides and a lower content of iso-α acids (**Table 3**), while addition of PGA contributed to its foam stability. It has been reported that this foam additive will not impart satisfactory head to beers that are deficient in foaming polypeptides, and our results are in agreement (*3*).

With the aim of establishing a model to evaluate the influence of hydrophobic polypeptides, iso-α acids, and malto-oligosaccharides on foam stability, we applied a discriminant analysis of the results obtained for beers without foam additives. The foam stability was used as grouping variable. For that purpose, samples 1−8 were divided into three groups according to their foam collapse time. The total contents of hydrophobic polypeptides, iso-α acids, and malto-oligosaccharides were the independent variables. Results from discriminant analysis are presented in **Tables 4** and **5** and in **Figure 4**.

Table 4 lists the standardized coefficients of the two canonical discriminant functions obtained for discriminant analysis. These two functions explain 100% of the variance, as is indicated by the eigenvalues. The significance of the total content of hydrophobic polypeptides in the two functions is notable, as well as the contents of total iso-α acids in the first function. This model suggests that these two variables in combination govern foam stability. In general, the foam stability decreased with decreasing contents of hydrophobic polypeptides and iso-α acids. Our results are in accordance with previous reports (*19*, *22*, *30*) that focus on the content of hydrophobic polypeptides as the key parameter. However, the role of iso-α acids appears to be pivotal, because brand 5 with a high content of hydrophobic polypeptides but a low content of iso-α acids showed very poor foam stability. Apparently, malto-oligosaccharides do not have a major impact on beer foam stability, in agreement with the view that oligosaccharides and polysaccharides are not foam-enhancing (*31*, *32*).

Table 5. Classification Results Obtained from Discriminant Analysis[a]

			predicted group membership			
		foam stability	1.00	2.00	3.00	total
original	count	1.00	3	0	0	3
		2.00	1	5	0	6
		3.00	0	3	15	18
	percentage	1.00	100.0	0	0	100.0
		2.00	16.7	83.3	0	100.0
		3.00	0	16.7	83.3	100.0
cross validated[b]	count	1.00	3	0	0	3
		2.00	1	5	0	6
		3.00	0	3	15	18
	percentage	1.00	100.0	0	0	100.0
		2.00	16.7	83.3	0	100.0
		3.00	0	16.7	83.3	100.0

[a] 85.2% of original grouped cases correctly classified, and 85.2% of cross-validated grouped cases correctly classified. [b] Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

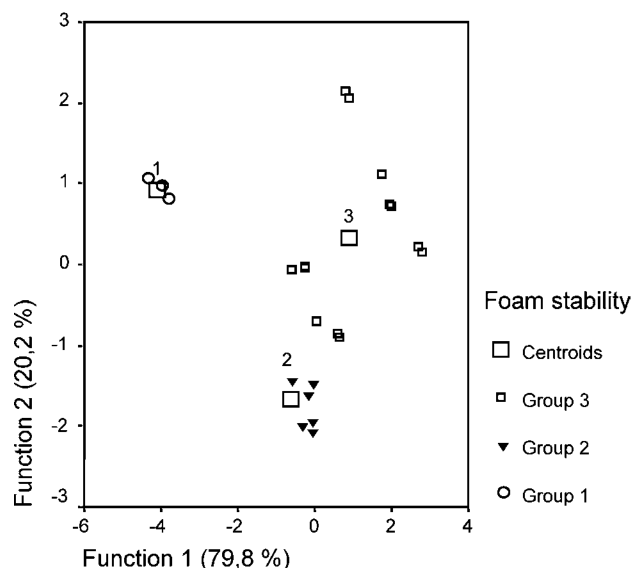

Figure 4. Canonical discriminant functions showing three groups of different foam stability classified by the two functions of the proposed model.

Lewis and Lewis when studying correlation of beer foam with other beer properties found strong correlations between total polypeptides, bitterness, polyphenols, real extract, total carbohydrates, viscosity, metal ions, and foam stability (*33*). However, as authors point out, a strong statistical correlation between two analytical parameters does not necessarily imply a cause-and-effect relationship, but it adds weight (or not) to parameters that reasonably could be or have already been related to foam stability.

Discriminant analysis was used to classify cases into the values of a categorical dependent (foam stability) and was effective for a set of data. The classification table of correct and incorrect estimates of 8 beers (without PGA) analyzed in triplicate with this model is presented in **Table 5** and **Figure 4**. The success rate was 85.2%, suggesting that foam stability is mainly governed by the beer constituents evaluated in this study. However, an incorrect classification was observed for groups 2 and 3, indicating that foam stability is stimulated by other beer constituents that were not investigated in the present study (**Table 5**). **Figure 4** shows the different groups according

to foam stability that were classified by the two functions of the proposed model.

In conclusion, elevated levels of barley-derived hydrophobic polypeptides influenced foam stability favorably; however, the presence of minimum levels of hop-derived iso-α acids is mandatory to obtain a stable foam (e.g., brand 5, with poor foam, low iso-α acid, and high polypeptide contents). Malto-oligosaccharides seem to have little impact on foam stability. The model presented here should be confirmed by a large-scale investigation of beers having widely divergent properties.

LITERATURE CITED

(1) Evans, D. E.; Sheehan, M. C. Don't be fobbed off: The substance of beer foam—A review. *J. Am. Soc. Brew. Chem.* **2002**, *60*, 47−57.

(2) Anger, H. M.; Glaro, L.; Scholz, M. A new process for foam stability measurement. *Brauwelt Int.* **2002**, *3*, 146−150.

(3) Bamforth, C. W. The foaming properties of beer. *J. Inst. Brew*. **1985**, *91*, 370−383.

(4) Blom, J. Investigations on foam. *J. Inst. Brew.* **1937**, *43*, 251−262.

(5) Constant, M. A practical method for characterizing poured beer foam quality. *J. Am. Soc. Brew. Chem.* **1992**, *50*, 37−47.

(6) Helm, E. Ueber eine Methode zur Schaumbestimmung. *Wochenschr. Brau.* **1933**, *5*, 241−243.

(7) Rudin, A. D. Measurement of the foam stability of beers. *J. Inst. Brew*. **1957**, *63*, 506−509.

(8) Hallgren, L.; Rosendal, I.; Rasmussen, J. A. Experiences with a new stability analyzer, Sustem Calsberg. *J. Am. Soc. Brew. Chem.* **1992**, *49*, 78−86.

(9) Jorge, K.; Nothaft, A.; Trugo, L. C. Concept of a foam analysis system using machine vision technologies. *Tech. Q. Master Brew. Assoc. Am*. **2003**, *40*, 108−110.

(10) Skands, B.; Lavrsen, L.; Bonfils, L. The use of a robot in assessing foam quality. *Monogr. Eur. Brew. Conv.* **1999**, *27*, 62−68.

(11) Hegarty, P. K.; Cope, R.; Crompton, I. E.; Wallach, E. R. An image analysis system for measuring beer foam stability. *Eur. Brew. Conv., Proc. Congr.* **1991**, 465−472.

(12) Yasui, K.; Yokoi, S.; Shigyo, T.; Tamaki, T.; Shinotsuka, K. A Customer-oriented approach to the development of a visual and statistical foam analysis**.** *J. Am. Soc. Brew. Chem.* **1998**, *56*, 152−158.

(13) Simpson W. J.; Hughes P. S. Stabilization of foams by hop-derived bitter acids. Chemical interations in beer foam. *Cerevisia Biotechnol.* **1994**, *19*, 39−44.

(14) Curioni, A.; Pressi, G.; Furegon, L.; Peruffo, D. B. Major proteins of beer and their precursors in barley: Electrophoretic and immunological studies. *J. Agric. Food Chem.* **1995**, *43*, 2620−2626.

(15) Hejgaard, J.; Kaersgaard, P. Purification and properties of the major antigenic beer protein of barley origin. *J. Inst. Brew.* **1983**, *89*, 402−410.

(16) Asano, K.; Hashimoto, N. Isolation and characterization of foaming proteins of beer. *J Am. Soc. Brew. Chem.* **1980**, *38*, 129−137.

(17) Sorensen, S. B.; Bech, L. M.; Muldbejerg, M.; Beenfeldet, T.; Breddam, K. Barley lipid transfer protein 1 is involved in beer foam formation. *Tech. Q. Master Brew. Assoc. Am.* **1993**, *30*, 136−145.

(18) Jégou, S.; Douliez J. P.; Mollé, D., Boivin, P.; Marion, D. Purification and structural characterization of LTP1 polypeptides from beer. *J. Agric. Food Chem.* **2000**, *48*, 5023−5029.

(19) Lusk, L. T.; Goldstein, H.; Ryder, D. Independent role of beer proteins, melanoidins, and polysaccharides in foam formation. *J. Am. Soc. Brew. Chem.* **1995**, *53*, 93−103.

(20) Slack, P. T., Bamforth, C. W. The fractionation of polypeptides from barley and beer by hydrophobic interaction chromatoghraphy: The influence of their hydrophobicity on foam stability. *J. Inst. Brew.* **1983**, *89*, 397−401.

(21) Onishi, A.; Proudlove, M. O. Isolation of beer foam polypeptides by hydrophobic interaction chromatography and their partial characterization. *J. Sci. Food Agric.* **1994**, *65*, 233−240.

(22) Leiper, K. A.; Stewart, G. G.; McKeown, I. P. Beer polypeptides and silica gel: Polypeptides involved in foam formation. *J. Inst. Brew*. **2003**, *109*, 73−79.

(23) Asano, K.; Hashimoto, N. Contribution of hop bitter substances to head formation of beer, Rep. Research Labs. *Kirin Brewery* **1976**, *19*, 9−16.

(24) De Keukeleire, D. Fundamentals of beer and hop chemistry. *Quim. Nova* **2000**, *23*, 108−112.

(25) Verzele, M.; De Keukeleire, D. *Chemistry and Analysis of Hop and Beer Bitter Acids*, Elsevier: Amsterdam, The Netherlands, 1991.

(26) Hughes, P. S.; Baxter, E. D. *Beer Quality, Safety, and Nutritional Aspects*, Royal Society of Chemistry: Cambridge, U.K., 2001.

(27) American Society of Brewing Chemists. *Methods of Analysis*, 7th ed., Beer 22A σ value method. The Society: St. Paul, MN, 1976.

(28) Jorge, K.; Trugo, L. C. Quality control of beer hopped with reduced isomerised products. *Arch. Latinoam. Nutr.* **1998**, *48*, 242−246.

(29) Nogueira, L. C.; Silva, F.; Ferreira, I. M. P. L. V. O.; Trugo, L. C. Separation and quantification of beer carbohydrates by high-performance liquid chromatography with evaporative light scattering detection. *J. Chromatogr., Sect. A* **2005**, *1065*, 207−210.

(30) St. John Coghlan, D.; Woodrow, J.; Bamforth, C. W.; Hinchliffe, E. Polypeptides with enhanced foam potential. *J. Inst. Brew*. **1992**, *98*, 207−213.

(31) Lusk, L. T.; Duncombe, G. R.; Kay, S. B.; Navarro, A.; Ryder, D. Barley β-Glucan and beer foam stability. *J. Am. Soc. Brew. Chem*. **2001**, *59*, 183−186.

(32) Hardwick, B. C.; Donley, J. R. Characterization of beer polysaccharides by enzymes and gel permeation chromatography. *J. Am. Soc. Brew. Chem*. **1977**, *35*, 29−32.

(33) Lewis, J.; Lewis, A. S. Correlation of beer foam with other beer properties. *Tech. Q. Master Brew. Assoc. Am.* **2003**, *40*, 114−124.

Received for review December 29, 2004. Revised manuscript received April 5, 2005. Accepted April 20, 2005.

JF047796W