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Communication

Determination of Osmolality in Beer to Validate Claims of Isotonicity

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Abstract: Alcohol-free beer is increasingly marketed with the claim “isotonic”. According to the European Food Safety Authority (EFSA), isotonic beverages should have an osmolality in a range of 270–330 mOsmol/kg. A method to determine osmolality in beer using an automatic cryoscope was applied and validated in this study. Isotonic and hypotonic beers can be measured directly, while hypertonic beers have to be diluted into the linear range of the instrument. As proven in several different beer matrices, the assay was linear with an average correlation coefficient of 0.998. The limits of detection and quantitation were 2 and 10 mOsmol/kg, so that the sensitivity of the method was judged sufficient to control the isotonic range. The measurement uncertainty expressed as coefficient of variation was less than 1% interday. The applicability of the method was proven by measurement of 86 beer samples. Our study has shown that the cryoscopic method is fit for the purpose to validate claims of isotonicity in food control.

Keywords: beer; nutritional claims; health claims; isotonic; osmolality; cryoscopy

1. Introduction

Isotonic sports beverages are available on the market aimed at restoring the strength of people conducting strong physical exercise [1,2]. Such drinks are usually used during or after exercise to avoid or delay the depletion of the body's carbohydrate stores and the onset of dehydration [3,4]. In recent years, isotonic alcohol-free beers were increasingly advertised and offered for the same purpose [5]. Alcohol-free beer (defined in Germany as beer with an alcoholic strength of less than 0.5% vol) may be a good alternative to traditional sports drinks or isotonic sports drinks, because its taste, which corresponds to normal beer, may be considered by some consumers as preferable to conventional sports drinks. Indeed, some studies have proven that many consumers may not even be able to differentiate alcohol-containing from alcohol-free beers [6–8]. Alcohol-free beer is the only segment of the German beer market that is currently increasing [9,10] and more and more types of alcohol-free beer are labeled with the claim "isotonic".

Consumers were found to have an increasing interest in the relationship between nutrition and health, and recent legislative efforts in Europe, such as the health claims regulation 1924/2006/EC or the food information legislation 1169/2011/EC, have strengthened the demands for appropriate labeling and scientific confirmation of labeling and marketing claims in this area [11]. For all these reasons, food control institutions need an analytical method to validate the nutritional claim "isotonic", most preferably in a rapid, easy and cheap fashion. Currently there is no reference method available for this purpose. Most methods in the literature were published in the 1980s or 1990s [12–19].

The osmolality is typically defined as the number of particles per kilogram of solvent water (mOsmol/kg H_2O) or by the International Union of Pure and Applied Chemistry (IUPAC) as quotient of the negative natural logarithm of the rational activity of water and the molar mass of water [20]. According to Egle *et al.* [21], isotonic drinks are classified as:

- Isotonic: 290 mOsmol/kg ± 15% (250–340 mOsmol/kg);
- Hypotonic: <250 mOsmol/kg;
- Hypertonic: >340 mOsmol/kg.

According to the Scientific Committee on Food (SCF) of the European Commission [22], drinks intended to meet the expenditure of intensive muscular effort, especially for sportsmen, should be formulated to cover a range of osmolalities between 200 and 330 mOsmol/kg. Beverages with an osmolality of 300 mOsmol/kg are isotonic. The SCF demands a stricter tolerance than Egle *et al.* [21], so that only beverages within a ±10% deviation (270–330 mOsmol/kg) may be designated as "isotonic". The German governmental expert committee, Arbeitskreis lebensmittelchemischer Sachverständiger (ALS) der Länder und des Bundesamtes für Verbraucherschutz und Lebensmittelsicherheit, agreed with SCF and also demands the strict range of 270–330 mOsmol/kg for claims of isotonicity [23]. Finally, the European Food Safety Authority (EFSA) suggested the same range in their evaluation of the health claim "isotonic". The proposed wording for the claims, which may only be used for foods for sportspeople under the directive 89/398/EEC, were "isotonic", "in balance with the body's own fluid", "isotonic drinks rapidly empty from the gut and promote water absorption", and "isotonic drinks help maintain hydration" [24].

The terminology associated with calculating and measuring osmotic activity is often confusingly used in the literature. The selection of which term to use (osmolality or osmolarity) depends on how the concentration was measured (see Equations (1) and (2)). When derived by an osmometer in clinical laboratories that use a method such as freezing point depression of water (or less commonly, the vapor pressure technique), the concentration is expressed in terms of solvent and is appropriately referred to as osmolarity [25]. In the case of beverages, the requirements of SCF, ALS and EFSA are stated in osmolality expressed in mOsmol/kg [22–24], so this unit is most typically used.

$$Osmolarity\left(\frac{mOsmol}{L\,H_2O}\right) = Osmolality \cdot \frac{Water\ content\ in\ solution\ in\ mL}{Volume\ of\ solution\ in\ mL} \tag{1}$$

$$Osmolality\left(\frac{mOsmol}{kg\,H_2O}\right) = Freezing\ point\ (°C) \cdot \frac{1000}{-1.86} \tag{2}$$

(Note: The freezing point depression of a molar solution is −1.86 °C.)

In this study, we applied and validated the freezing point methodology to confirm the claims of isotonicity in a collective of beer samples from the German market.

2. Experimental Section

An automatic cryoscope Cryostar II LC (Funke Gerber, Berlin, Germany) was applied with interface for printer and PC. A sample volume of 2.0 mL was measured in each case. The sample vials were provided by the manufacturer. The measurement begins about 2 °C below the freezing temperature [26]. Due to vigorous beating of the stirring bar against the glass wall, the sample suddenly crystallizes (freezing is triggered). The crystallization energy is released and warms the sample to its "freezing plateau" [26,27]. During the coexistence of the two phases, solid and liquid, the freezing temperature remains constant. After reaching this coexistence conditions, the freezing temperature is measured on this plateau with high reliability [27].

The instrument was calibrated for three different temperatures, using the following standard solutions:

- Standard A: freezing point at 0.000 °C ± 0.002 °C (bidistilled water).
- Standard B: freezing point at −0.514 °C ± 0.002 °C (0.8654 g of NaCl in 100 mL of bidistilled water).
- Standard C: freezing point at −0.557 °C ± 0.002 °C (0.9473 g of NaCl in 100 mL of bidistilled water).

All beverage samples were purchased from supermarkets in Baden-Württemberg, Germany. The analysis of each sample was performed in triplicate. The osmolality was calculated for each replicate, and the average of all replicates is reported. The degassed beer samples were measured either directly, or after dilution with water. A dilution was made when the direct measurement of the beer was outside the linear range of the instrument (0.000 °C to −1.000 °C). The osmolality was then calculated, taking into account the dilution factor of the sample solution.

Furthermore, a complete method validation was conducted. For validation the following samples were prepared and analyzed:

- 10 samples of isotonic alcohol-free beer, at 10 dilutions between 0% and 10% (total *n* = 100).
- 10 samples of standard beer (4%–6% vol alcohol), at 10 dilutions between 0% and 10% (total *n* = 100).
- 2 samples of an alcohol-free isotonic sports drink, at 10 dilutions between 0% and 10% (total *n* = 20).

To assess the intra- and interday precision, a random sample of beer was analyzed 6 times on a single day and on the following 5 days (total $n = 30$). To study the applicability of the method, all beer samples available at our laboratory in January 2015 were analyzed. For this reason, most samples were alcohol-containing standard beer types, because isotonic alcohol-free beers still have a comparably low market share on the German beer market.

3. Results and Discussion

During measurement of 86 samples, it was found that the freezing point in the range of isotonicity is well in the linear measurement range of the cryoscope when the beverages are directly measured, *i.e.*, without dilution. Only for most standard or strong beer types, which are typically hypertonic, a dilution into the measuring range may be needed. A problem with standard and strong beers may also be the ethanol content of the samples, which may hinder the instrument to reach the required temperature for measurement due to its low freezing point [28]. However, it should be noted that the measurement of normal and strong beers is not relevant for the question of labeling, because alcohol-containing beers may not be labeled as "isotonic" according to regulation 1924/2006/EC (which prohibits any health claim and most nutritional claims for alcoholic beverages).

The results of a total of 86 samples is shown in Table 1. In general, our results confirm previous literature regarding the tonicity of beer types, e.g., that standard beers are in the hypertonic range above 1000 mOsmol/kg [3,12,13,29]. Some strong beers, which so far have not been analyzed in the literature, may reach values up to 1600 mOsmol/kg. Our results also confirmed that alcohol-free beers may indeed fall into the isotonic range, even in some brands that were not labeled as isotonic. Most alcohol-free beers, however, are slightly hypotonic. Two of the product labeled as "isotonic" were outside of the strict range proposed by SCF, ALS and EFSA [22–24], but on the limits of the range of Egle *et al.* [21]. This also confirms observations from Switzerland that the osmolality of 35 commercial sports drinks from Switzerland tended to be outside the isotonic range [3]. Our institute will follow up such cases and increasingly include isotonic beverages in the official sampling for food control purposes.

Table 1. Osmolality and tonicity in a collective of different beer types.

Sample	Osmolality (mOsmol/kg), $n = 3$	Tonicity [a]	Type of drink [b]
1	253 ± 1	Hypotonic	Isotonic alcohol-free beer
2	344 ± 2	Hypertonic	Isotonic alcohol-free beer
3	272 ± 0	Isotonic	Isotonic alcohol-free beer
4	287 ± 1	Isotonic	Alcohol-free beer
5	244 ± 1	Hypotonic	Alcohol-free beer
6	232 ± 0	Hypotonic	Alcohol-free beer
7	171 ± 1	Hypotonic	Alcohol-free beer
8	216 ± 1	Hypotonic	Alcohol-free beer
9	229 ± 0	Hypotonic	Alcohol-free beer
10	258 ± 2	Hypotonic	Alcohol-free beer
11	273 ± 2	Isotonic	Alcohol-free beer
12	260 ± 0	Hypotonic	Alcohol-free beer

[a] Tonicity judged according to own analysis. All samples >500 mOsmol/kg were measured diluted; [b] According to labeling.

Table 1. *Cont.*

Sample	Osmolality (mOsmol/kg), *n* = 3	Tonicity [a]	Type of drink [b]
13	273 ± 0	Isotonic	Alcohol-free beer
14	457 ± 0	Hypertonic	Alcohol-free malt beverage
15	487 ± 0	Hypertonic	Alcohol-free malt beverage
16	493 ± 1	Hypertonic	Radler beer mix (shandy)
17	1096 ± 7	Hypertonic	Radler beer mix (shandy)
18	708 ± 3	Hypertonic	Radler beer mix (shandy)
19	1157 ± 17	Hypertonic	Standard beer
20	1060 ± 31	Hypertonic	Standard beer
21	1193 ± 52	Hypertonic	Standard beer
22	1232 ± 17	Hypertonic	Standard beer
23	1185 ± 5	Hypertonic	Standard beer
24	1018 ± 5	Hypertonic	Standard beer
25	1073 ± 0	Hypertonic	Standard beer
26	1095 ± 4	Hypertonic	Standard beer
27	1092 ± 5	Hypertonic	Standard beer
28	1073 ± 4	Hypertonic	Standard beer
29	1075 ± 1	Hypertonic	Standard beer
30	1066 ± 3	Hypertonic	Standard beer
31	1241 ± 7	Hypertonic	Standard beer
32	1176 ± 6	Hypertonic	Standard beer
33	1171 ± 13	Hypertonic	Standard beer
34	1168 ± 2	Hypertonic	Standard beer
35	994 ± 11	Hypertonic	Standard beer
36	1159 ± 2	Hypertonic	Standard beer
37	997 ± 4	Hypertonic	Standard beer
38	1100 ± 8	Hypertonic	Standard beer
39	1147 ± 10	Hypertonic	Standard beer
40	1067 ± 9	Hypertonic	Standard beer
41	1010 ± 1	Hypertonic	Standard beer
42	1071 ± 4	Hypertonic	Standard beer
43	1058 ± 8	Hypertonic	Standard beer
44	1074 ± 2	Hypertonic	Standard beer
45	1074 ± 1	Hypertonic	Standard beer
46	1122 ± 6	Hypertonic	Standard beer
47	1160 ± 9	Hypertonic	Standard beer
48	1050 ± 4	Hypertonic	Standard beer
49	1123 ± 6	Hypertonic	Standard beer
50	1054 ± 8	Hypertonic	Standard beer
51	1151 ± 7	Hypertonic	Standard beer
52	1071 ± 1	Hypertonic	Standard beer
53	1109 ± 8	Hypertonic	Standard beer
54	1105 ± 8	Hypertonic	Standard beer

[a] Tonicity judged according to own analysis. All samples >500 mOsmol/kg were measured diluted; [b] According to labeling.

Table 1. Cont.

Sample	Osmolality (mOsmol/kg), *n* = 3	Tonicity [a]	Type of drink [b]
55	1191 ± 5	Hypertonic	Standard beer
56	1158 ± 2	Hypertonic	Standard beer
57	1083 ± 2	Hypertonic	Standard beer
58	1159 ± 9	Hypertonic	Standard beer
59	975 ± 1	Hypertonic	Standard beer
60	1075 ± 1	Hypertonic	Standard beer
61	1136 ± 5	Hypertonic	Standard beer
62	1366 ± 5	Hypertonic	Standard beer
63	1109 ± 5	Hypertonic	Standard beer
64	1160 ± 8	Hypertonic	Standard beer
65	1025 ± 6	Hypertonic	Standard beer
66	1007 ± 6	Hypertonic	Standard beer
67	829 ± 7	Hypertonic	Standard beer
68	988 ± 2	Hypertonic	Standard beer
69	1110 ± 0	Hypertonic	Standard beer
70	970 ± 7	Hypertonic	Standard beer
71	982 ± 2	Hypertonic	Standard beer
72	975 ± 3	Hypertonic	Standard beer
73	1391 ± 8	Hypertonic	Standard beer
74	1066 ± 2	Hypertonic	Standard beer
75	1020 ± 9	Hypertonic	Standard beer
76	988 ± 3	Hypertonic	Standard beer
77	1018 ± 8	Hypertonic	Standard beer
78	709 ± 3	Hypertonic	Standard beer
79	949 ± 5	Hypertonic	Standard beer
80	1026 ± 6	Hypertonic	Standard beer
81	1125 ± 4	Hypertonic	Standard beer
82	1557 ± 5	Hypertonic	Strong Beer
83	1602 ± 12	Hypertonic	Strong Beer
84	1646 ± 15	Hypertonic	Strong Beer
85	1477 ± 4	Hypertonic	Strong Beer
86	1445 ± 5	Hypertonic	Strong beer

[a] Tonicity judged according to own analysis. All samples >500 mOsmol/kg were measured diluted; [b] According to labeling.

For validation purposes, dilution series of 22 samples were prepared to study the detection limits and linearity. The results are shown in Table 2. The assay was linear with an average correlation coefficient of 0.998. The average limits of detection and quantitation were 2 and 10 mOsmol/kg. The sensitivity of the method is therefore sufficient for official control purposes, because the limits are less than a factor of 10 lower than the lower limit of the isotonicity range (270 mOsmol/kg).

Table 2. Linearity and limits of detection and quantification calculated for aqueous dilution series of 22 beer samples.

Sample	Tonicity [a]	Type of drink [b]	Correlation coefficient	LOD [c] (mOsmol/kg)	LOQ [c] (mOsmol/kg)
1	Hypotonic	Isotonic alcohol-free beer	0.998	2	7
2	Isotonic	Isotonic alcohol-free beer	0.998	2	7
3	Isotonic	Alcohol-free beer	0.998	2	7
4	Hypotonic	Alcohol-free beer	0.998	2	6
5	Hypotonic	Alcohol-free beer	0.999	2	5
6	Hypotonic	Alcohol-free beer	0.996	2	6
7	Hypotonic	Alcohol-free beer	0.997	2	8
8	Hypotonic	Alcohol-free beer	0.997	2	7
9	Hypotonic	Alcohol-free beer	0.997	3	8
10	Hypertonic	Alcohol-free beer	0.998	2	8
11	Hypotonic	Alcohol-free beer	0.997	3	8
12	Isotonic	Isotonic alcohol-free sports drink	0.998	2	7
13	Isotonic	Isotonic alcohol-free sports drink	0.998	2	7
14	Hypertonic	Standard beer	0.999	4	15
15	Hypertonic	Standard beer	0.999	7	23
16	Hypertonic	Standard beer	0.999	4	14
17	Hypertonic	Standard beer	0.999	3	11
18	Hypertonic	Standard beer	0.999	3	12
19	Hypertonic	Standard beer	0.999	3	12
20	Hypertonic	Standard beer	0.999	5	18
21	Hypertonic	Standard beer	0.999	4	15
22	Hypertonic	Standard beer	0.999	4	13
Average	-	-	0.998	3	10

[a] Tonicity judged according to own analysis; [b] According to labeling; [c] LOD: Limit of detection; LOQ: limit of quantification.

For final method validation, the intraday and interday precision were determined by consecutive measurement of a quality control sample over several days (Table 3). The coefficient of variation (CV) was below 0.5% (intraday) and below 1% (interday), which shows an excellent precision of the method, compared to typical chromatographic or spectroscopic methods in food analysis (e.g., for additives or contaminants) that may reach CVs as high as 15% and are still judged as acceptable (e.g., see guidelines in Shah *et al.* [30]). The standard deviation (in absolute units) during application to authentic samples (Table 1) was typically below 2 mOsmol/kg in the hypotonic and isotonic range, and below 10 mOsmol/kg in the hypertonic range, due to the additional error of the necessary dilution.

Table 3. Intra and interday precision of osmolality determination in beer.

Replicate	Day 1	Day 2	Day 3	Day4	Day 5	Overall [a]
1	1073	1098	1075	1075	1095	-
2	1073	1087	1067	1071	1098	-
3	1073	1091	1073	1075	1090	-
4	1074	1087	1066	1074	1085	-
5	1075	1085	1073	1073	1085	-
6	1074	1089	1073	1071	1090	-
Mean	1073	1089	1071	1073	1090	1080
SD	0.8	4.6	3.7	1.8	5.2	9.2
CV (%)	0.08	0.43	0.35	0.17	0.48	0.86

[a] The overall statistics were calculated for all 30 measurements in total.

4. Conclusions

In contrast to other nutritional claims such as carbohydrates, fat, protein or alcohol, which need to be validated by complex instrumental methods such as NMR spectroscopy [31], time-consuming wet-chemical [11] or physicochemical methods [32], the claim of isotonicity can be checked rather rapidly using a simple cryoscopy measurement. Cryoscopes are also already available in many food-testing laboratories, because it has been a standard method in milk analysis for decades [33,34]. As our research shows, the methods established for milk analysis can be used for beers and alcohol-free beverages with minimal modification (such as dilution if necessary). Our validation has shown that the method is fit for the purpose of food control. Despite the controversial nature of the use of the isotonicity claim on beverages that appear to be targeted to the general consumer, when the benefits are only expected in the small sub-group of heavy athletes [1–3], we believe that it is still important to regularly confirm the validity of this claim to protect the consumers from misleading food information.

Author Contributions

DWL conceived of the study. JLLT performed the experiments, analyzed the data and wrote the first manuscript draft. DWL participated in data analysis and drafting the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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