XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos
Alimentação: a árvore que sustenta a vida
X CIGR Section IV International Technical Symposium
Food: the tree that sustains life
24 a 27 de outubro de 2016 • FAURGS • GRAMADO/RS

RECOMMENDATIONS FOR GRADUAL SUGAR REDUCTION IN PROBIOTIC CHOCOLATE-FLAVOURED MILK

D.C.R. Oliveira[1], R. Deliza[2], F.R. Reis[3], A. Rosenthal[2], A. Giménez[4], G. Ares[4]

1- Instituto de Química, Universidade Federal do Rio de Janeiro, Av. Athos da Silveira Ramos 149, Cidade Universitária – CEP: 21949-909 – Rio de Janeiro – RJ – Brazil, e-mail: (denize.cris@hotmail.com)
2 - Embrapa Agroindústria de Alimentos, Av. das Américas, 29501 – CEP 23.020-470, Rio de Janeiro – RJ – Brazil, E-mail: (rosires.deliza@embrapa.br; amauri.rosenthal@embrapa.br)
3 - Departamento de Tecnologia de Alimentos, Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, BR 465 Road 7 km – CEP: 23890-000 – Seropédica – RJ – Brazil, e-mail: (felipereis86@yahoo.com.br)
4 - Sensometría & Ciencia del Consumidor, Instituto Polo Tecnológico de Pando, Facultad de Química, Universidad de la República. By Pass de Rutas 8 y 101 s/n, C.P. 91000. Pando, Canelones, Uruguay (gares@fq.edu.uy)

RESUMO – O trabalho visa fornecer recomendações para a redução gradual de açúcar no leite achocolatado por meio da determinação dos limiares de diferença de doçura para o açúcar adicionado e avaliar a percepção sensorial e hedônica dos consumidores dos produtos com açúcar-reduzido. Cinco estudos foram realizados (50 consumidores/estudo) para determinar cinco limiares de diferença sequenciais. Em cada estudo foram realizados seis testes de comparação pareada. Cada par foi composto de um leite probiótico achocolatado controle e uma amostra com redução de açúcar em relação ao controle. Limiares de diferença correspondentes à menor redução na concentração de açúcar percebida pelos consumidores foram determinados usando análise de sobrevivência. Em seguida, foi realizado um estudo com 100 consumidores para avaliar a percepção sensorial e hedônica das amostras com diferentes quantidades de açúcar adicionado. Os resultados sugerem que reduções de açúcar sequenciais podem ser fixadas em 6,66% sem afetar a percepção sensorial e hedônica dos consumidores, constituindo-se alternativa viável e de fácil implementação pela indústria.

ABSTRACT – The work aimed at providing recommendations for gradual sugar reduction in chocolate-flavored milk by determining difference thresholds for added sugar and evaluating consumers' sensory and hedonic perception of reduced-sugar products. Five studies were conducted (50 consumers/study) to determine five sequential difference thresholds. In each study, consumers completed six paired-comparison tests. Each pair was composed of a probiotic control chocolate-flavored milk and a sample that was reduced in added sugar from the control. Difference thresholds, corresponding to the smallest reduction in sugar concentration perceived by consumers were determined using survival analysis. Then, a study with 100 consumers was carried out to evaluate the sensory and hedonic perception of chocolate-flavored milk samples with different added sugar. Results suggested that sequential sugar reductions could be set at 6.66% without affecting consumers' sensory and hedonic perception, which is an alternative feasible and easy to implement.

PALAVRAS-CHAVE: análise de sobrevivência; comparação pareada; CATA; estudos com consumidores.

KEYWORDS: survival analysis; paired-comparison; CATA; consumer studies.

1. INTRODUCTION

Humans prefer sweet taste since the newborn stage. Generally, children and adolescents have high acceptance for higher concentrations of sucrose than adults do. Sugar has become a major hidden source of calories and its intake has been strongly associated with the growing prevalence of several negative health conditions such as obesity, type 2 diabetes and dental caries (Morenga et al., 2013). This situation requires the development of strategies to reduce sugar consumption worldwide (Lustig et al., 2012).

Considering the contribution of added sugar to our daily energy intake, one of the most realistic strategies that can be implemented to gradually reduce sugar consumption is to reduce the concentration of sugar added by the food industry (MacGregor & Hashem, 2014). This type of strategy has been successfully implemented in the UK for reducing salt consumption (Wyness et al., 2011). According to the English Department of Health, a reduction of 30 to 40% in the added sugar can reduce on average 100 kcal/day/person, which could be effective in preventing obesity and diabetes (Department of Health, 2011).

In this context, the present work aimed at providing recommendations for gradual sugar reduction in a probiotic chocolate-flavoured milk by determining difference thresholds for added sugar and evaluating consumers' sensory and hedonic perception of reduced-sugar products.

2. MATERIAL AND METHODS

2.1. Formulation of probiotic chocolate-flavored milk

Chocolate-flavored milk samples were formulated using UHT whole milk (Conaprole, Uruguay), commercial sugar ranging from 9.0% to 5.93% (Alcoholes del Uruguay S.A., Bella Unión, Uruguay), 2.5% alkaline cocoa powder (ARYES Aroma and Essences, Montevideo, Uruguay), 0.08% carrageenan (Ticaloid® 780 Stabilizer - Texture Innovation Center, TIC GUMS, Philadelphia, USA) and 0.1% freeze-dried probiotic culture of *Lactobacillus acidophilus* (Nu-trish® LA-5® Freeze-dried - CHR HANSEN, Denmark). Probiotic chocolate-flavored milk samples were prepared as described by Oliveira et al. (2015) and stored under refrigeration temperature (4 °C ± 1 °C).

2.2. Estimation of difference thresholds

Five sequential difference thresholds were determined as follows: in the first study, difference threshold for sugar in probiotic chocolate-flavoured milk with an added sugar concentration similar to commercial products available in the Uruguayan marketplace (9%). In the second study the difference threshold for sugar in a probiotic chocolate-flavoured milk that was reduced in added sugar according to the threshold determined in the first study was estimated. This pattern was repeated until five difference thresholds were determined.

The studies involved 250 consumers (68% female; from18 to 29 years old). Each of the five studies was carried out with a group of 50 consumers. All participants were recruited among students of the Universidad de la República (Montevideo, Uruguay) according to their frequency of product consumption (at least once a week), interest and availability. In each study, consumers completed six paired-comparison tests. Each pair was composed of a control chocolate-flavoured milk, different control samples were used in each study, and a sample that was reduced in added sugar from the control. The control remained constant in each study, while the sugar reduced sample increased over the series of paired-comparison. The sugar concentration of the control and the sugar-reduced samples

in each of the studies is shown in Table 1. Sugar concentrations in each of the studies were selected by pilot testing.

Table 1 - Sugar concentration and sugar-reduction percentage (between brackets) of probiotic chocolate-flavored milk samples considered in the five studies in which sequential difference thresholds for sugar were determined.

Study	Control sample %	Sugar reduced samples (%)					
		1	2	3	4	5	6
1	9.00	8.70 (3.3)	8.40 (6.7)	8.10 (10.0)	7.80 (13.3)	7.50 (16.7)	7.20 (20.0)
2	8.30	8.05 (3.0)	7.80 (6.0)	7.55 (9.0)	7.30 (12.0)	7.05 (15.1)	6.80 (18.1)
3	7.80	7.60 (2.6)	7.30 (6.4)	7.10 (9.0)	6.80 (12.8)	6.60 (15.4)	6.30 (19.2)
4	7.25	7.00 (3.4)	6.80 (6.2)	6.50 (10.3)	6.30 (13.1)	6.00 (17.2)	5.80 (20.0)
5	6.83	6.68 (2.2)	6.53 (4.3)	6.38 (6.6)	6.23 (8.8)	6.08 (11.0)	5.93 (13.2)

Consumers were asked to taste each of the samples in a pair and to select the sweeter one by choosing the corresponding number using Compusense-at-hand (Compusense Inc., Guelph, Canada). Samples in each pair were presented following a balanced design. Testing took place in a sensory laboratory designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22 ºC).

2.3. Consumers' sensory and hedonic perception of probiotic chocolate-flavoured milks with different added sugar concentration

A study was carried out to evaluate consumers' sensory and hedonic perception of probiotic chocolate-flavored milk samples with different added sugar concentrations. Nine samples were considered, corresponding to the reference samples of the five studies (9.00; 8.30; 7.80; 7.25; and 6.83% of added sugar) performed in the difference threshold studies, the sample with the sugar concentration determined in Study 5 (6.40%) and three additional samples. The added sugar concentration of these three last samples was intermediate between the concentration of the control sample and the reduced ones, according to the threshold in Studies 1, 3 and 5.

One hundred consumers participated in the test (74% female; 18-25 years old). They were recruited following procedure mentioned in the previous section. Consumers were asked to try the samples and to indicate their overall liking using a 9-point hedonic scale (1: dislike very much, 9: like very much) and to answer a check-all-that-apply (CATA) questions composed of nine sensory characteristics: bitter, rough, chocolate, thick, sweet, fluid, greasy, milk flavour, and vanilla, which were identified in previous studies using open-ended questions. Samples were presented following a balanced order of presentation (Williams' Latin Square design). Data were collected using Compusense at-hand (Compusense Inc., Guelph, Ontario, Canada). Testing took place in a sensory laboratory, as described in the previous section.

Difference thresholds were estimated using survival analysis, following a modification of the procedure proposed by Alcaire et al. (2014). Survival analysis methodology was used to determine the difference threshold in each of the studies. Difference thresholds for each sample were determined as

the sugar-reduction percentage at which 50% of the consumers had their difference thresholds. Calculations were performed using the R scripts provided by Hough (2010).

The overall liking was analyzed by ANOVA. Sample was specified as fixed effect, whereas consumer was specified as a random effect. Tukey's test was used for post hoc pair wise comparisons of sample means, at a significance level of 5%.

The frequency of use of each CATA term was determined by counting the number of consumers who used that term to describe each sample. Cochran's Q test (Manoukian, 1986) was carried out separately to identify significant differences among samples on each of the sensory terms.

All data analyses were carried out using R software version 3.1.1 (R Core Team, 2014).

3. RESULTS AND DISCUSSION

When survival analysis was used to model the percentage of consumers having difference thresholds lower than each sugar-reduction percentage, the lognormal distribution showed the best fit in all studies. As an example, Figure 1 shows the percentage of assessors with difference thresholds lower than each g sugar-reduction percentage. As shown, a good fit was obtained for the experimental data.

Figure 1. Percentage of consumers with difference threshold equal or lower than each sugar reduction percentage from the Study 1, in which the control sample was formulated with 9% added-sugar.

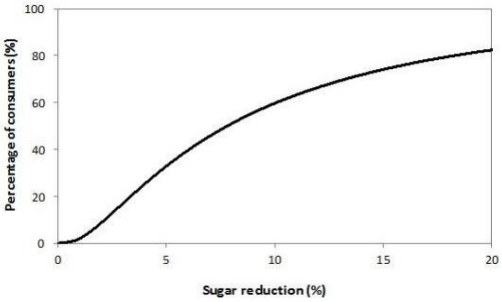

Table 2 shows the difference thresholds for each of the studies and their corresponding confidence intervals. As shown, difference thresholds ranged from 5.74% to 7.77% of the added sugar concentration of the control sample. According to the 95% confidence intervals difference thresholds did not significantly change when the added sugar concentration of the control sample changed, suggesting that sugar reductions in the different studies could be regarded as a constant proportion of the original stimulus. Across the five studies the Weber fraction corresponded to an average of 6.66% of the added sugar concentration of the control sample.

Table 2. Difference thresholds* and 95% confidence intervals for added sugar in probiotic chocolate-flavored milks with respect to references with different added sugar concentration in five consumer studies.

Study	Added-sugar concentration of the control (%)	Difference threshold[§] (%)	Sugar reduction from the control sample (%)	95% confidence interval
1	9.00	8.30	7.77	5.59-10.80
2	8.30	7.80	6.31	4.66-8.53
3	7.80	7.25	6.99	4.77-10.26
4	7.25	6.83	5.74	4.11-8.02
5	6.83	6.38	6.47	4.87-8.59

[§] expressed as added sugar concentration

Table 3. Overall liking scores and percentage of consumers who used each of the terms of the CATA question for nine probiotic chocolate-flavoured milk samples with different added sugar concentration.

Parameter	Sugar concentration (%)								
	9.00#	**8.50**	**8.30#**	**7.80#**	**7.40**	**7.30#**	**6.80#**	**6.50**	**6.40#**
Overall liking	5.9[a]	6.1[a]	6.1[a]	6.0[a]	5.7[a]	5.9[a]	5.7[a]	5.6[a]	5.4[a]
Chocolate *	76	74	72	74	78	74	68	62	50
Sweet *	90	86	72	64	68	58	54	48	36
Fluid [ns]	48	36	44	42	50	42	50	54	56
Milk flavour [ns]	36	38	38	36	38	46	38	50	60
Thick [ns]	34	44	30	42	38	40	34	26	20
Bitter *	12	22	22	38	26	34	38	38	42
Vanilla [ns]	32	34	28	24	26	20	22	28	22
Greasy [ns]	24	24	30	28	28	24	22	26	20
Rough [ns]	18	22	24	20	16	16	34	28	30

[#] Samples included in the difference threshold study. Identical superscripts indicate that average overall liking scores are not significantly different according to Tukey test ($p>0.05$).
* The frequency of use of CATA question terms significantly differed among samples ($p<0.05$), whereas [ns] revealed that they did not significantly differ among samples.

Sugar reduction did not significantly affect overall liking scores ($p=0.08$). Average overall liking scores ranged from 5.4 to 6.1 for all samples, as shown in Table 3.

Regarding the sensory characteristics of samples, significant differences in the frequency of use of the terms of the CATA question among samples with different sugar concentration were only identified for the attributes chocolate flavor, sweetness and bitterness. Sugar reduction caused a significant decrease in the frequency of use of the terms chocolate flavor, and sweet, and a significant increase in the frequency of use of the term bitter. Although CATA questions do not directly measured attribute intensity, Ares et al. (2015) showed that the frequency of use of CATA terms tends to be linearly correlated with attribute intensity. Therefore, results from the present work indicate that a reduction from 9.00% to 6.40% in added sugar concentration in probiotic chocolate-flavored milk caused a decrease in sweetness and chocolate flavor intensity, as well as an increase in bitterness intensity. It is interesting to note that changes in the frequency of use of the above mentioned terms were observed at different sugar reduction percentages.

As expected, samples that differed in less than the difference threshold (9.00% vs. 8.50%, 7.80% vs. 7.40% and 6.80% vs. 6.50%) were perceived as very similar by consumers, which confirms that sugar reductions smaller than the difference threshold would not affect consumers' sensory perception of the products. Five sequential difference thresholds for sugar were determined, ranging from 9.00% to 6.83%. Difference thresholds did not significantly differ for the five control products, which support Weber's law. This indicates that added sugar concentration can be reduced an average of 6.66%, regardless of the specific sugar concentration of the probiotic chocolate-flavored milk.

Reducing added sugar in probiotic chocolate-flavored milk from 9.00% to 6.40% led to changes in three sensory product characteristics: sweetness, chocolate flavor and bitterness. Although these changes were particularly relevant between samples with the highest and lowest added sugar concentration, when differences in sugar concentration were equal or larger than the difference threshold consumers tended to perceive differences in their sensory characteristics.

Interestingly, no significant differences in overall liking were found among samples with different added sugar concentration. Therefore, a reduction of 28.9% in added sugar did not cause a significant change in consumers' hedonic perception of probiotic chocolate-flavored milk. This result is in agreement with Oliveira et al. (2015), who reported that reducing 20% added sugar did not cause significant differences in liking in probiotic chocolate-flavored milk. According to their results, a decrease of 30% in added sugar in strawberry and coffee flavoured yogurts caused a significant

decrease in overall liking. These results suggest that a recommendation for sequential sugar reduction is product specific.

4. CONCLUSION

Results suggest that sugar reduction in probiotic chocolate-flavored milk without affecting consumers' product perception seems feasible and easy to implement. Considering a constant Weber fraction of 6.66%, added sugar concentration in one-year time can be reduced 12.9% by implementing two sequential reductions. If this same strategy were implemented during three years would allow meeting the recommendations of the UK Department of Health to reduce added sugar by 30-40%. In the case of the probiotic chocolate-flavored milk samples considered in the present study, this reduction would not lead to a significant change in consumers' sensory and hedonic perception.

The approach of the present work could be implemented to design recommendations for gradual reduction of the added sugar concentration of other commercial dairy products, as well as products targeted to children (e.g. fruit juices) contributing to the development of more healthful products that meet current nutritional recommendations.

5. ACKNOWLEDGEMENTS

The authors are indebted to Comisión Sectorial de Investigación Científica (Universidad de la República, Uruguay), CAPES-Brazil and CNPq, Brazil for financial support.

6. REFERÊNCIAS BIBLIOGRÁFICAS

Alcaire, F., Zorn, S., Cadena, R.S., Antúnez, L., Vidal, L., Giménez, A., & Ares, G. (2014). Application of survival analysis to estimate equivalent sweet concentration of low-calorie sweeteners in orange juice. *Journal of Sensory Studies*, 29, 474-479.

Ares, G., Antúnez, L., Bruzzone, F., Vidal, L., Giménez, A., Pineau, B., et al. (2015). Comparison of sensory product profiles generated by trained assessors and consumers using CATA questions: Four case studies with complex and/or similar samples. *Food Quality and Preference*, 45, 75-86.

Department of Health. (2011). *Healthy lives, healthy people: a call to action on obesity in England*. London: Department of Health.

Hough, G. (2010). Sensory shelf life estimation of food products. Boca Raton, FL:Taylor & Francis.

ISO. (2007). *Sensory Analysis: General Guidance for the Design of Test Rooms, ISO 8589.* Geneva, Switzerland: International Organization for Standardization.

Lustig, R. H., Schmidt, L. A., & Brindis, C. D. (2012). Public health: The toxic truth about sugar. *Nature*, 482, 27-29.

MacGregor, G.A., & Hashem, K.M. (2014). Action on sugar—lessons from UK salt reduction programme. *Lancet*, 383, 929-931.

Morenga, L., Mallard, S., & Mann, J. (2013). Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ*, 346, e7492.

Oliveira, D., Antúnez, L., Giménez, A., Castura, J. C., Deliza, R., Ares, G. (2015). Sugar reduction in probiotic chocolate-flavored milk: Impact on dynamics sensory profile and liking. *Food Research International*, 75, 148-156.

R Core Team (2014). R: *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Wyness, L.A., Butriss, J.L., & Stanner, S.A. (2011). Reducing the population's sodium intake: the UK Food Standards Agency's salt reduction programme. *Public Health Nutrition*, 15, 254-261.