APPLICATION OF WHEY-DERIVED SYRUPS IN DAIRY PRODUCTS

V. Somov[a], I. Evdokimov[b,*], S. Knyazev[a], S. Perminov[a], Yu. Kurash[c]

[a] Wimm Bill-Dann/Dairy R&D,
Dmitrovskoe Shosse 108, Moscow, 127591 Russian Federation

[b] North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355009 Russian Federation

[c] PepsiCo/Long Term Research,
Skyline Drive 3, Hawthorne, NY, 10532 USA

* e-mail: ek-v-b@yandex.ru

Received April 24, 2015; Accepted in revised form May 14, 2015; Published October 20, 2015

Abstract: Sugar substitution is a hot topic in current food and beverage development. Sugar substitute is a food additive with sugar-like taste and usually cheaper than sugar. We developed production of glucose-galactose syrup (GGS) from cheese whey to replace and lower sucrose content in dairy products. Nanofiltrated whey containing 15% lactose underwent enzymatic and demineralization processing, producing different levels of monosaccharaides and electrolytes. We hypothesized that the amount of glucose/galactose and minerals in GGS might mediate sweet taste transduction resulting in different perception of sweetness. Using cell-based approach we demonstrated a link between GGS composition, cellular response, and sensory data. GGS with 20% glucose and 16% galactose activated sweet taste transduction and had similar sweetness level compared to sucrose. Moreover, demineralization level of GGS mediated sweet perception and cellular responses. Taken together, our results provide opportunities to optimize production at low-cost GGS from whey to reduce sugar in various PepsiCo products.

Keywords: Glucose-galactose syrup (GGS), whey, lactose, nanofiltration, demineralization, dairy products

DOI 10.12737/13113 *Foods and Raw Materials*, 2015, vol. 3, no. 2, pp. 89–95.

INTRODUCTION

Sweet taste plays a critical role in the recognition of food and nutrition, and maintaining energy homeostasis. The sweet taste signals from presence of carbohydrates in solution triggers a pleasurable response. Over the past four decades, sugar substitute is the fastest growing segment of the sweetener market. A sugar substitute is a food additive that duplicates the effect of sugar in taste, usually with reduced calories.

Whey is a by-product resulting from dairy industry especially cheese production. It contains a good amount of a disaccharide, lactose that upon hydrolysis yields glucose and galactose leading to increased sweetness (Fig. 1).

Conversion of glucose to fructose and galactose to tagatose further increases sweetness and decrease calories (Fig. 1). Therefore, whey-derived syrups might be used as a sugar substitute.

We produced glucose-galactose syrups (GGS) with different amounts of monosaccharaides and electrolytes. We proposed that GGS composition may regulate sweet signaling pathways leading to different sweetness level. Recent molecular studies have revealed that the sweet receptor heterodimer T1R2/T1R3 is responsive to sweet tasting compounds [1]. Sweet ligands bind to the T1R2/T1R3 receptor and activate G-protein pathway transduction, which include receptor internalization, activation of secondary messengers and intracellular calcium mobilization [2]. Several studies demonstrate that there are T1R-independent mechanisms for sweet taste signaling [3, 4]. It was shown that T1R3-positive taste cells express glucose transporter GLUT4 [3] and glucagon receptor [4] suggesting that these signaling proteins may serve as mediators of sweet taste.

Recently, we have demonstrated that various sweet-tasting compounds selectively activate multiple receptors leading to different perception of sweetness [5]. Natural sugars activate T1R2/T1R3-mediated signaling cascades, whereas artificial sweeteners target both sweet receptors T1R2/T1R3 and GLUT4. Non-caloric sugars, i.e. rebaudioside A activate additional receptor, glucagon receptor, which mediates sweetness. Importantly, HFCS targets four receptors: T1R2, T1R3, GLUT4, and glucagon, indicating that activation of multiple signaling cascades is responsible for HFCS sweetness.

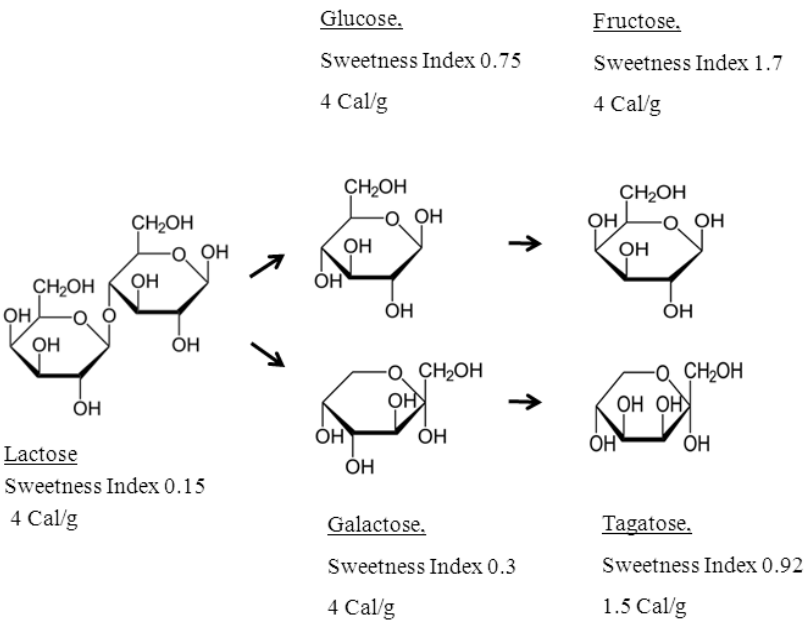

Fig. 1. Chemistry of lactose conversion to useful sweet molecules.

Using cell-based approach and bench top ranking sensory test we demonstrated that the amount of lactose in GGS mediated sweet taste transduction resulting in different perception of sweetness. Thus, GGS with 15% lactose, 20% glucose, and 16% galactose led to activation of T1R2/T1R3 and glucagon receptors mediating sweetness; whereas GGS with 20% lactose, 8% glucose, and 5% galactose significantly increased GLUT4 internalization resulting in aftertaste. Additionally, a correlation was observed between the demineralization level of GGS, receptor-recycling routes, and sensory data. Bench-top sensory studies demonstrated that GGS with low concentration of lactose and 70% demineralization did not affect sweetness in dairy products with 25% sugar reduction, thereby opening doors for utilization of a waste stream to deliver cost-effective sugar reduction in PepsiCo products.

OBJECTS AND METHODS OF STUDY

Production of GGS

GGS production from whey was conducted using baromembrane method. Dry whey was comprised of protein (0.73%), lactose (4.45%), fat (0.05%), and ash (0.55%). First, the whey underwent an ultrafiltration process that reduced the protein and fat content. Concentrated whey contained 0.03% protein, 4.4% lactose, 0% fat, and 0.5% ash. Then, whey was three-times concentrated down until the brix reached 19%. The final concentrations of whey components in nanofiltrated (NF) concentrate were 17% lactose, 1% protein, and 0.4% ash. NF concentrate was treated with β-galactosidase for four hours at 40°C to hydrolyze lactose into glucose and galactose at similar concentrations. Finally, NF concentrate was evaporated under vacuum at 0.8 bars until Brix reached 65–70%. Fig. 2 shows the process of GGS production from whey.

Chemical and sugar analysis

Chemical analysis of whey components and sugar concentration were determined using the following methods: (a) potentiometric method (pH measurement); (b) refract metric method (Brix measurement); (c) Duma's method (protein concentration); (d) enzymatic method (concentration of lactose, galactose, and glucose); (e) digestion at +520°C (ash content); (f) atomic absorption (cation content, i.e. K^+, Na^+, Ca^{2+}, Mg^{2+}); (g) IC method (anions content).

Materials for cell-based assay

Rabbit anti-T1R2 and rabbit anti-T1R3 antibodies were from Thermo Fisher. Rabbit anti-GLUT4, mouse anti-Glucagonreceptor, andrabbitanti-GLP1Rantibodies were from Sigma. Alexa488-conjugated antibodies and Hoechst 33342 were from Life Technology. NCI-H716 cell line was purchased from ATCC.

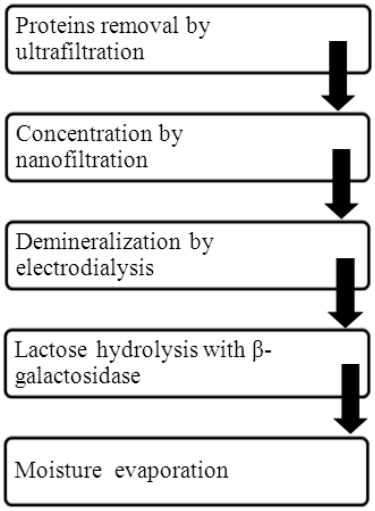

Fig. 2. Production of GGS from whey.

Cell-based assay

NCI-H716 cells were grown in RPMI1640 supplemented with 10% fetal bovine serum. Cells were seeded at density of 20 000 cells/well on PDL-coated 384-well plates. Then cells were treated with GGS at 6.5–7.0% brix or with control sweet molecules at 100 mM.

High-Content imaging and analysis

Cells were fixed in 4% formaldehyde and then permeabilized in 0.5% Triton X-100 in DPBS. For GLUT4 and Glucagon receptor staining cells were fixed in methanol/acetone. Primary antibody was added to each well for 16 h at +4°C. After washing the cells three times with D-PBS, secondary Alexa488-conjugated antibody was added for 30 min at room temperature. Nuclear staining was performed using Hoechst 33342 and incubated at room temperature for 15 minutes. Images were acquired using an ImageXpress Micro automated epifluorescence microscope (Molecular Devices Corporation). Images were analyzed with MetaXpress 4.0 Workstation software, utilizing the Multiwaves Translocation Scoring analysis algorithm for nuclear and cytoplasmic segmentation (Fig. 3). "Ring" positive cells were calculated by correlation coefficient for pixel values of Hoechst 33342 and Alexa 488 (receptor) signals [6]. Curve fitting and parameter estimation were analyzed with TIBCO Spotfire.

Bench top ranking sensory test

All sensory analyses were performed in Dairy R&D panel, using untrained employee personnel. Bench top yogurts were prepared using sucrose (100%), sucrose (75%), and 75% sucrose with GGS (Fig. 4). These experiments were replicated more than three times. Ranking analysis of sweetness was conducted as there are no perceivable sensory differences between exist sample and samples with 75% of sucrose with GGS.

RESULTS AND DISCUSSION

GGS composition

The disposal of whey remains a significant problem for the dairy industry. One possible approach to whey utilization is hydrolysis of lactose resulting in glucose-galactose syrup which might be used as a sucrose substitute. We developed production of GGS from NF concentrate using enzymatic approach. The lactose hydrolysis reactions were carried out using a commercial β-galactosidase. GGS generated from cheese whey contained varying amounts of monosaccharaides and lactose, depending on the β-galactosidase enzymatic activity. Produced GGS can be divided into two major types based on the level of lactose hydrolysis (Table 1). GGS type 1 contained high amount of monosaccharaides (20% glucose and 15% galactose), and 14% lactose, whereas type 2 had low amount of glucose-galactose (8% glucose and 5% galactose) and 20% lactose (Table 1).

We conducted to examine the effects of lactose hydrolysis level on sweet taste transduction using cell-based assay and sweetness level in dairy desserts with 25% sucrose reduction.

Cell-based assay results

Receptor internalization upon stimulation with ligand is considered to be a key component of a cellular response [1]. To determine whether GGS stimulates

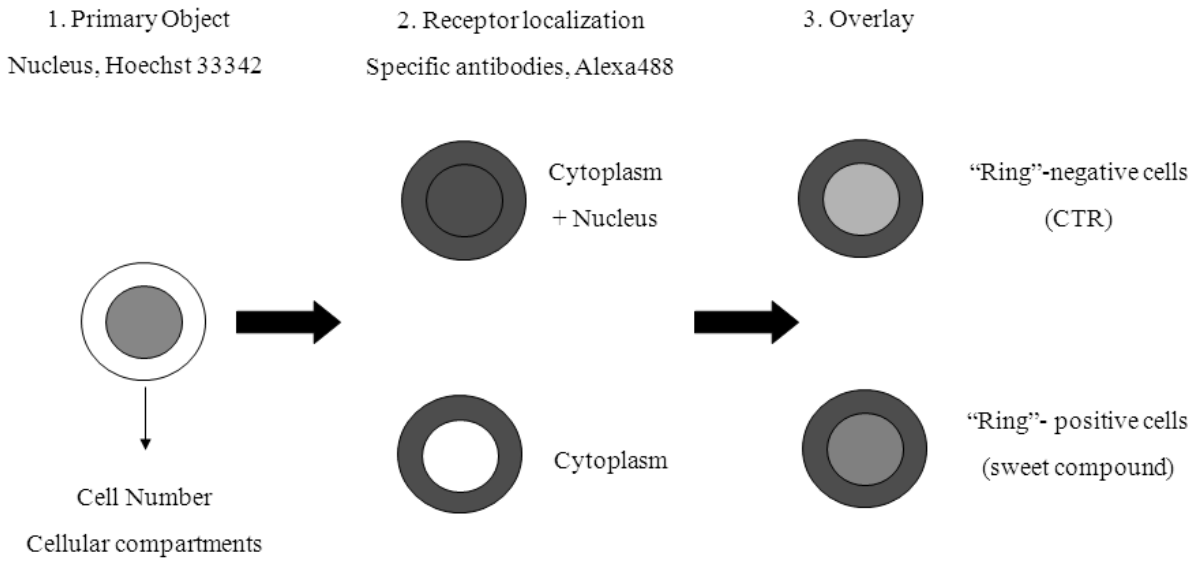

Fig. 3. Image analysis algorithm for quantification of "Ring"-positive cells.

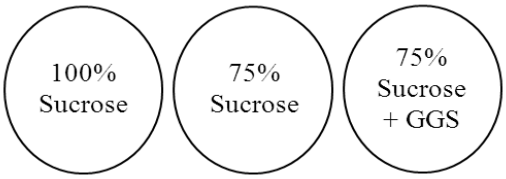

Fig. 4. Bench-top ranking sensory test.

internalization of receptors underlying sweet taste transduction, we developed a High-Content imaging assay using the human enteroendocrine L cell line NCI H716 that respond to sweet compounds [7].

First, we investigated the effects of GGS with various degrees of lactose hydrolysis on sweet receptor T1R2/T1R3 internalization. Recent molecular studies have revealed that the sweet receptor heterodimer T1R2/ T1R3 is responsive to sweet tasting compounds and activate G-protein pathway transduction [1]. We have demonstrated that untreated NCI-H716 cells expressed T1R2/T1R3 receptors at the cell-surface (Fig. 5, left). Treatment with sweet-tasting compounds induced T1R2/T1R3 receptor internalization and trafficking T1R2/T1R3 receptor internalization and trafficking from the target membrane to cytosolic vesicles, resulting in typical "Ring"-staining (Fig. 5, right). Using Molecular Devices Multiwaves Translocation Scoring Module, we quantitated internalization of endogenous T1R2/T1R3 in NCI-H716 cells treated with GGS (Table 2).

We have found that T1R2/T1R3 internalization increased after stimulation with GGS type 1 (Table 2). In contrast, GGS type 2 had a strong effect on T1R3 internalization only. Sample #1 of GGS type 2 slightly induced T1R2 internalization, whereas even minimal T1R2 internalization did not occur upon treatment with sample #2 of GGS type 2 (Table 2). Intriguingly, several other studies demonstrated distinct contributions of T1R2 and T1R3 taste receptor subunits in detection of sweet stimuli [8]. It was found that T1R3 requires co-expression with T1R2 to form a fully functional sweet taste receptor, whereas homomeric T1R3 receptor may act as low-efficacy sugar receptors [8]. Therefore, our data have demonstrated a link between GGS composition and activation of different subunits of sweet taste receptor.

Table 1. GGS composition (sugar)

Syrup type	Sample #	Glucose, %	Galactose, %	Lactose, %
1	1	21.1	17.0	13.7
	2	20.8	15.2	14.7
2	1	8.9	5.9	20.6
	2	8.1	5.2	22.4

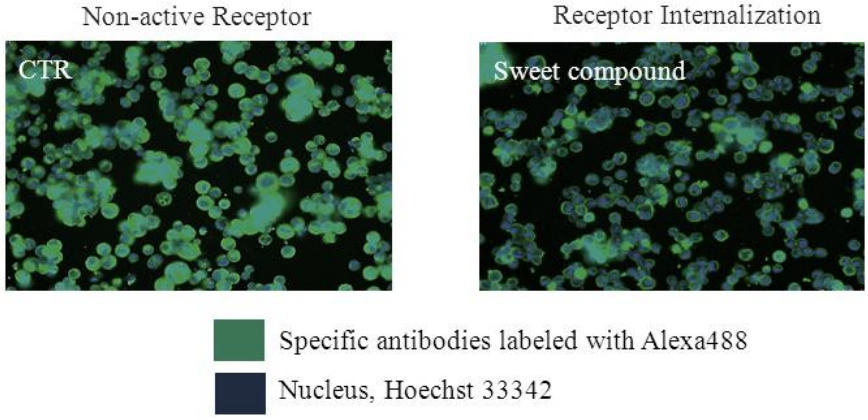

Fig. 5. "Ring"-formation assay.

Table 2. Link between GGS composition to cellular response and sensory

Syrup Type	GGS Samples				Cellular Response				Bench-top Sensory
	Sample #	Glucose, %	Galactose, %	Lactose, %	Sweet Receptor		Glucose Transporter GLUT4	Glucagon Receptor	
					T1R2	T1R3			
1	1	21.1	16.9	13.7	+	+	-	+	Sweetness match
	2	20.8	14.9	14.7	+	+	-	+	Sweetness match
2	1	8.3	5.2	22.4	+	+	++	-	Aftertaste
	2	8.9	5.9	20.6	-	+	-	+	Less sweet

Recently, we have demonstrated the existence of T1R-independent mechanisms for sweet taste signaling (personal communication). We showed that artificial sweeteners and non-caloric sugars, i.e. rebaudioside A activate GLUT4 and glucagon receptor, respectively in addition to sweet. GLUT4-mediated cellular response correlates with bitter and sweet aftertaste of artificial sweeteners, whereas glucagon receptor mediates sweetness (personal communication).

To further explore a link between GGS composition and cellular response, we investigated the internalization of GLUT4 and glucagon receptor in NCI-H716 cells. Both samples of GGS type 1 induced internalization of glucagon receptor and were not able to activate GLUT4 internalization (Table 2). In contrast, GGS type 2 robustly activated either GLUT4 internalization (sample #1), or slightly induced internalization of glucagon receptor (sample #2) (Table 2).

Recently, we have demonstrated that various sweet-tasting compounds selectively activated internalization of multiple receptors leading to different perception of sweetness (Table 3).

For example, glucose, or fructose activate T1R2 sweet receptor-mediated signaling cascade; galactose, or lactose stimulate GLUT4 and glucagon receptor internalization; treatment with sucrose leads to internalization of T1R2/T1R3 sweet receptor (Table 3). Interestingly, HFCS stimulates internalization of four receptors: T1R2, T1R3, GLUT4, and glucagon (Table 3), whereas mixture of 55% glucose+45% fructose induces internalization of T1R2 only, indicating that activation of multiple signaling cascades is responsible for HFCS sweetness.

Similar to HFCS, GGS type 1 activated multiple receptors, T1R2/T1R3 sweet receptor and glucagon receptor mediating sweet perception. In contrast, we did not observed a specific profile in cellular response upon treatment with GGS type 2. Taking together, our data demonstrated a relationship between level of lactose hydrolysis in GGS and activation of sweet taste signaling pathways.

Bench-top sensory data

To determine whether GGS composition and activation of specific sweet taste transduction receptors are responsible for GGS sweetness, we tested GGS type 1 and type 2 in bench-top ranking sensory studies. Experimental bench top yogurts were made using 100% sucrose, 75% sucrose, and 75% sucrose with GGS in fruit preparation recipe. In general, bench top yogurts with GGS type 1 were similar in sweetness level compared to control yogurts (Table 2). In contrast, bench top yogurts with GGS type 2 were significantly different compared to reference samples and possessed sweet aftertaste (Table 2). Thereby, sensory analysis data correlated with GGS composition and cellular response, providing useful experimental approach for further optimization of GGS production with different sensory properties.

Electrolytes composition in GGS

NF concentrate contains varying amounts of non-sugar substances, e.g. ash. During evaporation from 15 brix to 65–70 brix, there is a risk of scale formation. To prevent scale forming in the evaporation station and prevent precipitation during storage, a portion of the minerals have been removed by electrodialysis. Using GGS with ~24% glucose, ~20% galactose, and 0–2.9% lactose, we prepared GGS with 0%, 50% and 70% demineralization level (Table 4). Then, GGS samples were subjected to receptor internalization studies and sensory evaluation.

Cell-based assay and bench-top sensory results

Using High-Content imaging we have demonstrated that GGS with 0% and 70% demineralization levels, like HFCS activated four receptors, T1R2/T1R3, GLUT4 and glucagon (Table 5). In contrast, we did not observe

Table 3. Cellular response and sensory data, control samples

Control Samples	Sweet Receptor		Glucose Transporter GLUT4	Glucagon Receptor	GLP1 Receptor	Sweetness Index
	T1R2	T1R3				
Glucose	+	-	-	-	-	0.75
Galactose	-	-	+	+	-	0.3
Lactose	-	-	+	+	-	0.16
Sucrose	+	+	-	-	-	1.0
Fructose	+	-	-	-	-	1.7
45% Glucose+55% Fructose	+	-	-	-	-	<1.2
HFCS	+	+	+	+	+	1.2

Table 4. GGS composition (electrolytes)

Sample #	Sample name	Conductivity, mS	Ca^{2+}, mg/kg	Mg^{2+}, mg/kg	Na^{+}, mg/kg	K^{+}, mg/kg
1	Without demineralization	9	1 778	881	9 063	17 908
2	Demineralization 50%	3.8	1 365	563	2 613	3 760
3	Demineralization 70%	2.5	1 305	526	2 078	2 693

the effect on T1R2 internalization upon treatment with GGS with 50% demineralization level (Table 5), suggesting that GGS with 50% demineralization might have low sweetening potency and sweet aftertaste. Actually, bench-top sensory studies demonstrated that 50% demineralization of GGS affected sweetness level and possessed sweet aftertaste in dairy desserts with 25% sucrose reduction (Table 6).

At the same time, bench top yogurts with 0% and 70% demineralization of GGS were significantly different in sweetness level compared to each other (Table 6). GGS with 0% demineralization possessed sweet aftertaste, whereas GGS with 70% demineralization was similar in sweetness level compared to control yogurts and had like honey taste (Table 6).

Recently, we demonstrated a correlation between the molecular structures of sugars and T1R2-recycling routes. Thus, T1R2 recycled back to the cell membrane very quickly upon treatment with monosaccharaides, D- glucose and D-fructose, whereas slow T1R2-recycling pathway was observed with the disaccharide sucrose and its analog sucralose (personal communication). Moreover, we found that HFCS induced delayed T1R2/T1R3 sweet receptor internalization and slow T1R2/T1R3-recycling pathway compared to mixture of 55% glucose+45% fructose (Table 6), suggesting that internalization kinetics and recycling routes might be responsive for sweetness quality.

To explore the link between demineralization level of GGS, sweetness level and recycling kinetics of receptors mediating sweet taste signaling, we performed internalization kinetics studies. We found that GGS with 0% and 70% demineralization levels, like HFCS induced delayed T1R2/T1R3 sweet receptor internalization and slow T1R2/T1R3-recycling pathway (Table 6). However, we observed the significant difference in internalization kinetics and recycling routes for GLUT4 and glucagon receptor between GGS with 0% and 70% demineralization levels and HFCS. HFCS activated GLUT4 and glucagon receptor at 5 min and both receptors recycled back to the cell membrane at 15 min (Table 6). Treatment with GGS at 70% demineralization stimulated additional delay in GLUT4 and glucagon receptor internalization with quick recycling to the cell membrane, whereas GGS with 0% demineralization induced delayed internalization of GLUT4 and glucagon receptor and slow recycling pathway (Table 6).

Our results provided evidence that GGS with different sweetness level activated diverse patterns and kinetics of sweet taste signaling cascades and receptor trafficking routes, further supporting the conclusion that receptor internalization events mediate sweetness level of GGS and providing novel opportunities for optimization of GGS production from whey.

Practical application of whey-derived syrups

GGS was approved for use in Russia in various dairy products [9]. Table 7 shows the examples of fruits preparation recipes currently using in dairy desserts, such as mixed yogurts, drinkable yogurts and spoonable yogurts. Sucrose might be replaced with either HFCS or with GGS (Table 7). 25% replacement of sugar with GGS in fruit preparation recipe would result in saving of 6 MM USD per year. Application of GGS in dessert milk product recipes may bring additional 5 MM USD in savings.

Table 5. Link between demineralization level of GGS to cellular response (dose-response studies)

Sample #	GGS composition				Cellular response				Sensory
	Glucose, %	Galactose, %	Lactose, %	Demineraliza-tion level, %	T1R2	T1R3	GLUT4	Glucagon	
1	23.7	20.0	2.9	0	+	+	+	+	Sweet, Bitter aftertaste
2	24.4	15.9	0	50		+	+	+	Less sweet, Bitter aftertaste
3	26.3	21.6	2.6	70	+	+	+	+	Sweet, Honey taste

Table 6. Link between demineralization level of GGS to cellular response (kinetic study) and sensory

Sample #	Glucose, %	Galacto-se, %	Lactose, %	Demine-ralization level, %	Glucagon			GLUT4			T1R2			T1R3			Sensory
					5	15	30	5	15	30	5	15	30	5	15	30	
1	23.7	20.0	2.9	0		+	+		+	+		+	+		+	+	Sweet, Bitter aftertaste
2	24.4	15.9	0	50			+			+					+	+	Less sweet, Bitter
3	26.3	21.6	2.6	70			+			+		+	+		+	+	Sweet, Honey taste
HFCS					+	+		+	+			+	+		+	+	
45% Glucose + 55% Fructose											+			+			

Table 7. Sample of GGS application in fruit preparation recipe

Ingredients	Condition	kg/ton	kg/ton	kg/ton
Sugar	Bx 99	590.0	435.0	75.0
HFCS	Bx 71		219.0	0.0
GGS	Bx 75	0.0	0.0	690.5
Cranberry concentrate	Bx 64-66 TK (based on citric acid pH=8.1) 14.0–21.0%	40.0	40.0	40.0
Raspberry concentrate	Bx 64-66 TK (based on citric acid pH=8.1) 9.0–12.0%	27.0	27.0	27.0
Pectin	YM-115 H CP Kelco	4.0	4.0	4.0
Cranberry aroma	No. 321793 Symrise	6.0	6.0	6.0
Raspberry aroma	No. 648289 Symrise	1.0	1.0	1.0
Color carmine	9% Biocolor 180, Carmiliq, Naturex/Overseal	0.55	0.55	0.5
Water		331.45	267.45	156.00
Total		1 000.0	1 000.0	1 000.0

CONCLUSION AND NEXT STEPS

Taken together, we demonstrated a link between the amount of lactose/ monosaccharaides and minerals in GGS, cellular response, and sensory data. Additionally, we provided opportunity for PepsiCo to use GGS in dairy products. In general terms, replacing sugar with whey-based syrup, can produce savings with maintaining a high quality end-product. In the meantime, additional areas of commercial application of GGS will be explored.

For the next steps, optimization of enzymatic hydrolysis and demineralization process will be evaluated.

REFERENCES

1. Nelson G., Hoon M.A., Chandrashekar J., Zhang Y., Ryba N.J. and Zuker C.S. Mammalian sweet taste receptors. *Cell*, 2001, vol. 106, no. 3, pp. 381–390.
2. Linderman B. Receptors and transduction in taste. *Nature*, 2002, no. 413, pp. 219–225.
3. Yee K.K., Sukumaran S.K., Kotha R., Gilbertson T.A. and Margolskee R.F. Glucose transporters and ATP-gated K+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1R3)-expressing taste cells. *Proc. Natl. Acad. Sci. USA*, 2011, vol. 108, no. 13, pp. 5431–5436.
4. Elson A.E., Dotson C.D., Egan J.M. and Munger S.D. Glucagon signaling modulates sweet taste responsiveness. *FASEB J*., 2010, vol. 24, no. 10, pp. 3960–3969.
5. Somov V., Perminov S., Knyazev S., Kurash Y.K., Aglione A., Dragan S., Cassutt K. and Gravina S. Application of Whey-derived syrups in PepsiCo dairy products. *Global R&D Research Forum*. Chicago, 2013.
6. Kurash Y.K. and Gravina S. *Taste Receptor Internalization Assay*. Patent WO, no. 2014183041 A1, 2013.
7. Jang H.J., Kokrashvili Z., Theodorakis M.J., Carlson O.D., Kim B.J., Zhou J., Kim H.H., Xu X., Chan S.L., Juhaszova M., Bernier M., Mosinger B., Margolskee R.F. and Egan J.M. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc. Natl. Acad. Sci. USA*, 2007, vol. 104, no. 38, pp. 15069–15074.
8. Nie Y., Vigues S., Hobbs J.R., Conn G.L. and Munger S.D. Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. *Curr Biol*., 2005, vol. 15, no. 21, pp. 1948–52.
9. Somov V., Perminov S. and Knyazev S. *Fruit preparation with GGS, technology process*. Patent RF, no. 2011154292, 2013.

Please cite this article in press as: Somov V., Evdokimov I., Knyazev S., Perminov S. and Kurash Yu. Application of whey-derived syrups in dairy products. *Foods and Raw Materials*, 2015, vol. 3, no. 2, pp. 89–95. doi: 10.12737/13113.