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Literature report

Sugar reduction in yogurt: Technological possibilities and sensorial ob- servations

On behalf of the FSVO

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1 Summary

The following report consists of a compendium of review papers and scientific studies on sugar reduction, specifically focused on dairy products and particularly on yogurts. These articles discuss the role of sugar in product formulation, potential cross-modal interactions between flavor, texture and color occurring in a food matrix and their possible optimization, based on sensory and/or technological approaches.

An important strategy is the sequential or “silent” sugar reduction in which the reduction steps are chosen in such a way that the consumer cannot detect the difference between the product with the initial sugar concentration and the sugar-reduced product. The main advantage is that there is no reformulation needed. However, there is little knowledge on the number of possible reduction steps before consumers perceive a difference to the original product and product acceptance starts to decrease. Since the difference thresholds highly depend on the type of food matrix product specific reduction steps have to be determined. There is limited information on suggested time periods that need to be awaited before a next reduction step can be taken.

Most studies found in literature about sugar reduction focuses on sugar replacement. In yogurt, particularly high intensity sweeteners have been extensively tested. The main disadvantage of these sweeteners remains their different sensory quality and temporality compared to sucrose and their controversial effects on long-term health.

The addition of appropriate aromas allows an increase in the perceived sweetness intensity due to cross-modal interactions, but literature on this topic is scarce and the results of the various interactions are product-specific and difficult to predict.

The addition of protein to improve the yogurt texture is widely used in the dairy industry. This step could also offer a way to improve sweetness intensity of yogurts as sweetness may be influenced by the caseinate-whey protein ratio as well as type of production of whey concentrate. The addition of sweet peptides seems a promising strategy to reduce sugar in food products. However, there is still little research on their potential application in yogurt and there is no evidence on safety and health aspects

The specific choice of bacteria cultures used for yogurt production may represent another valid strategy to actively reduce the amount of added sugars in yogurt. Supporting the formation of compounds naturally occurring in yoghurt such as enzymes, peptides, glucose or aroma compounds may directly or indirectly (lower production of lactic acid) enhance the perceived sweetness of yogurt. However, scientific studies evaluating the metabolic pathways used by bacteria to form these desired compounds are still rare. In addition, the effect on sweetness enhancement by this strategy might be rather small.

Another proposed strategy to reduce sugar content in food is the use of multisensory integration principles, such as a combination of various approaches like texture modification, aroma addition, and food coloring. No study was found in the literature that evaluated the effect on sweetness perception of systematically combined known sensory cross modal interactions. Therefore, it is difficult to estimate the size of a possible sugar reduction by simultaneous application of the above-mentioned strategies.

2 Method of research, keywords

Literature research on sugar reduction in yogurt and dairy-based products encompassed peer reviewed scientific journals, books and patent searches and was conducted based on the following literature search providers:

- Scopus
- Pubmed
- Google Scholar
- ScienceDirect
- Espacenet
- Google Patents

The keywords used for the literature search on sugar reduction (typed in different combinations) are listed below:

- Amino acids
- Aroma
- Casein
- Coffee
- Consumer acceptance
- Culture
- Dairy
- Desserts
- Enhancement
- Fermentation
- Flavor / Flavor
- Interactions
- Milk
- Modulation
- Perception
- Protein
- Reduction
- Reformulation
- salt
- Sensitivity
- Sensory, sensory contrast
- Silent sugar reduction
- Starch
- Strawberry
- Sugars / Sugar / Sucrose / Saccharose / Syrup / Sweetener
- Sweetness
- Technology
- Texture
- Thresholds
- Whey
- Yogurt / Yogurt / Jogurt / Joghurt

3 Introduction

The impact of excessive sugar intake on health is currently one of the most discussed public health issues, which also includes regulatory aspects. Various studies have shown a positive correlation between a high sugar intake in the diet and the risk of pathologies such as obesity, diabetes type II, coronary heart diseases as well as caries. Therefore, decreasing sugar consumption is considered to be the most efficient and promising strategy to lower the risk for these health problems. Guidelines published in 2015 by the World Health Organization (WHO) on sugar consumption recommend a reduction of intake of “free” sugars (added mono/di-saccharides) to less than 10% of total energy intake. This corresponds to less than 50g sugar per day (WHO 2015).

Sugar reduction targets the organoleptic and chemical/physical characteristics of a wide range of food products and involves various approaches depending on the type of product investigated.

The aim of this report is to give an overview of the scientific literature on potentially new and already applied approaches for sugar reduction, focusing mainly on yogurt and other dairy products.

4 Definitions

4.1 Yogurt types

Yogurt is produced by bacterial fermentation of milk. The classic yogurt starter culture consists of the two symbiotic species of lactic acid bacteria *S. thermophilus* and *Lb. delbrueckii ssp. bulgaricus*. These microorganisms convert lactose into lactic acid, which is responsible for the acidic taste of yogurt. Thus, the pH is reduced to between 4.2 to 4.5 depending on strain composition of the starter culture. (Das, Choudhary, and Thompson-Witrick 2019). In addition to the acid, the lactic acid bacteria also produce typical yoghurt flavour components such as acetaldehyde, diacetyl and acetoin (Chen et al. 2017). A wide range of different types of yogurt is offered by food producers. Plain yogurt made from whole milk typically consists of 85.6% water, 4% protein, 3.6% fat, 3.6% lactose, and approximately 1% minerals and different vitamins (Sieber 2012). Stirred and set yogurts are the two main yogurt types produced in Switzerland. Stirred yogurts are obtained by stirring the yogurt after acidification before filled in containers sold on the market, while for the set products the acidification takes place directly in the yogurt container. The stirred technology is commonly used for fruit-containing yogurts, while set yogurt is mostly used for “brown” flavors such as coffee, chocolate and vanilla as well as fruit yogurts flavored only with fruit aroma molecules. Additionally, in the past years, yogurts with an increased protein content have appeared on the Swiss market.

4.2 Sugars

A multitude of terms is used to describe sugars or dietary mono- and di-saccharides and the definitions often differ between countries (Table 1).

Table 1: Definitions used for sugars in dietary recommendations (adapted from SACN (2015))

Term	Definition
Non-milk extrinsic sugars (NMES)* - UK 1991	Sugars not contained within the cellular structure of food except lactose in milk and milk products.
Intrinsic sugars - UK 1991	Intrinsic sugars are those naturally incorporated into the cellular structure of foods
extrinsic sugars – UK 1991	extrinsic sugars are those sugars not contained within the cellular structure of a food (i.e. lactose in milk and milk products)
Free sugars* - WHO 2015	Monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates (excludes lactose in milk and milk products)
Added sugars - US 2005	Sugars and syrups added to foods during processing and preparation. Added sugars do not include naturally occurring sugars such as lactose in milk or fructose in fruits
Added sugars – EFSA 2009	Sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing.
Total sugars - EFSA 2009	endogenous (sugars naturally present in foods such as fruit, vegetables, cereals and lactose in milk products) and added sugars

* Non-milk extrinsic sugars includes 50% of the fruit sugars from stewed, dried or canned fruit, but free sugars includes none.

4.3 “Added sugars”

Based on the definition given by the “European High Level Group on Nutrition and Physical Activity” the Federal Food Safety and Veterinary Office (FSVO) of Switzerland provided its own definition for ‘added sugars’. The term “added sugars” is specifically used to refer to “sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing. The term “added sugars” also includes sugars present in honey, syrups, fruit juices, fruit juice concentrates, and sugars from other foods with sweetening properties (e.g. fruit powder, fruit pulp or malt extract). Sugar alcohols (polyols) such as sorbitol, xylitol, mannitol, and lactitol, are usually not included in the term “added sugars”. The present definition is in agreement with the nutrition claim “with no added sugars” (EC-Regulation 2006), which is part of Swiss Food Law. The FSVO also highlights the difference between the term “added sugar” and “of which sugars”. The latter is often found on product labels in Switzerland in addition to the amount of carbohydrates. The term “of which sugars” refers to all mono- and disaccharides, which includes both ‘added sugars’ and sugars naturally present in food such as lactose in dairy products.

According to the FSVO there are three categories of ingredients that add sugar to food:

- Category 1: Ingredients consisting of sugars (100% sugars)
Glucose, dextrose, grape sugar, fructose, laevulose, d-tagatose, sucrose, saccharose, crystal sugar, refined sugar, icing sugar, candied sugar, rock sugar (from cane or beets, whole and crude), maltose, malt sugar, barley sugar, isomaltulose, caramel sugar, “aromatized” sugars (vanilla, vanillin, cinnamon sugars), etc.
- Category 2: Ingredients containing sugars (mono-/ disaccharides as ‘added sugars’)
Liquid sugar, sugar beet syrup and molasses, sugarcane syrup, sugars from fruits, starch syrup, glucose syrup, fructose syrup, high-fructose-corn syrup (HFCS), caramel syrup, honey, invert sugar, molasses, malt extract, concentrated fruit juice, syrups (fruits, agave, maple), fruit powder, pulps, purees, etc.
- Category 3: Composite ingredients containing ingredients of category 1 and/or 2
Sugared fruit pieces and cereal flakes, fruit jam, etc.

The declaration of “added sugars” on product labels is useful to provide reliable information about the actual amount of added sugar in the product as well as to facilitate monitoring and regulation of added sugars in the diet. However, in contrast to the US, it is not mandatory to declare “added sugars” in the EU/CH.

5 Sugar consumption in Europe and Switzerland

In Europe, sugars represent between 15 and 21% of the total energy intake of adults and approximately 16 to 26% of children. When only added sugars are considered, sugar accounts for 7 to 11% and 11 to 17% of daily energy intake of adults and of children, respectively, highlighting the major contribution of these sugars on the energy intake (Azaïs-Braesco et al. 2017). In Switzerland, the FSVO estimates that the average daily intake of sugars is 110 g per day, i.e. about double the amount recommended by the WHO (FSVO 2019b). Independent of age, gender and country, four main food categories are the major contributors of total sugar intake in Europe: sweet products (e.g. confectionery, chocolate, jam), fruit, beverages and dairy products (in order of importance) (Azaïs-Braesco et al. 2017). In Switzerland, the three main food sources of added sugars are sweet products (47%), beverages (29%) and dairy products (9%) with yogurt accounting for 6% (Chatelan et al. 2019). Although dairy products are considered as healthy products as they are important suppliers of essential nutrients such as calcium and vitamins yogurts are often sweetened with saccharose to balance the yogurt acidity.

For some years now, the Swiss Federal Government has set itself the goal of reducing the sugar content in products, which contain high amounts of added sugars and are consumed on a daily basis. The aim is to promote a healthy lifestyle and on the long run reduce the healthcare costs associated with a high sugar intake. In 2015, the “Milan Declaration”, an agreement aiming to gradually reduce the sugar content in yogurts and breakfast cereals, was signed by the Swiss government and leading food producers (FSVO 2019b).

In order to monitor the progress of the agreed sugar reduction, data on sugar content of fruit yogurts and flavored set yogurts as well as other products such as cereal is collected and analyzed annually in Switzerland. In 2016, the FSVO conducted a first survey on the sugar content of yogurt. The monitoring of 348 sweetened yogurts from seven companies showed that an average of 9.4 g sugars per 100 g are added to yogurts (min 5.5 g and max 17.0 g/100g) (Infanger 2017). The results of the survey conducted in 2018 showed a 4.3% decrease in the median added sugar content of fruit yogurts (median for added sugar in yogurt in 2016 = 9.2g/100g and for 2018=8.8g/100g) (FSVO 2019c).

A recent international study showed that in 2018 flavored yogurts contained an average of 12.4 g total sugars per 100 g in UK, 10.1g/100g in South Africa and 11.9 g/100g in Australia (Coyle et al. 2019).

6 The role of sugar in foods

Sugar is a major energy contributor to human nutrition. However; today, energy intake in the form of sugar consumption has become a major public health problem. Nevertheless, sugar is an important food ingredient due to its wide range of functionalities. It enhances flavor perception and viscosity and can act as a bulking and coloring agent, as well as a preservative. Furthermore, it is widely accepted that sweetness intensity is a major factor determining consumer acceptance of a high percentage of a wide range of food products. Thus, any reduction of the sugar content of a product should be done while trying to avoid changes of the sensory product characteristics which are crucial for consumer acceptance (Markey, Lovegrove, and Methven 2015).

The most important characteristics of sugars is their potential to evoke a sweet taste. As already mentioned sweetness intensity has a high impact is a major factor determining on consumer acceptance of a food product. Sugar-derived sweetness influences the perception of various other product characteristics. Especially the interaction between perceived sweetness and sourness is a well-known phenomenon in many foods and beverages (Baldwin, Goodner, and Plotto 2008; Keast and Breslin 2003).

Particularly in yogurt, the sensation of sourness that derives mainly from the activity of the lactic acid bacteria can be balanced by the addition of sugars.

Moreover, selected sugars have the potential to increase the intensity of specific aromas, such as strawberry (Pfeiffer et al. 2006), vanilla and caramel (Guinard and Mazzucchelli 1999) as well as citrus (Fujimaru and Lim 2013). In contrast, sweetness is interacting with other taste qualities for example by suppressing bitterness (Pineli et al. 2016) as well as saltiness (Gillan 1982).

Sugars have also a significant impact on the perception of food texture, especially for solid products. In particular, sugars have been shown to influence viscosity (Pangborn, Trabue, and Szczesniak 1973) and thickness (Oliveira et al. 2015), as well as structural characteristics of foods, such as emulsion stability (Maskan and Göğüş 2000), the unfolding and aggregation of proteins during heat treatment (Kulmyrzaev, Bryant, and McClements 2000), or gelatinization (Chantaro and Pongsawatmanit 2010).

Sugar also influences the color of a product. Especially when heated, sugar breaks down and can react with proteins (Maillard reaction) resulting in the color and desirable flavors that characterizes many cooked foods (Pareyt et al. 2009). In frozen fruits and jellies, sugar was shown to prevent unwanted color changes of the product such as enzymatic browning and fading of the color caused by water absorption (Varzakas, Labropoulos, and Anestis 2012).

From a food safety point of view, sugars play a role in food preservation. An increase in sugar concentration results in a decreased water activity that can inhibit microbial growth and, in consequence, extend the shelf life of a product (Farkas 2007; Smith et al. 2004).

7 Perception of sweetness

Sweet is together with salty, bitter, sour and umami one of the well-known taste qualities, perceptible by humans. According to recent research, there are also human taste receptors for a so-called fat taste (Mattes 2009). Evidence shows that humans have an inborn preference for sweetness. In addition, physiological factors such as age, sex and BMI also influence sweet taste perception (Jotterand Chaparro, Moullet, and Farina 2017). Technically, the term "taste" should only be used as a description for the pure gustatory properties (i.e. sweet, salt, sour, bitter). The mixture of taste and olfactory sensations that is perceived during the ingestion of most foods is called "flavor" and is used for the qualitative/quantitative sensory perception derived from the interaction between tastants and odorants (Rozin 1982).

Taste receptor cells (TRC) responsible for the perception of sweetness and other taste qualities are mainly located in the oral cavity but are also present in the gastrointestinal system (Iwatsuki et al. 2012). Physiologically, sweet, bitter and umami tastes are mediated by different G protein-coupled receptors (GPCRs), type 1 taste receptors (T1Rs) for sweet and umami, and type 2 taste receptors (T2Rs) for bitter taste. Type 1 taste receptor 3 (T1R3) subunit combines with the type 1 taste receptor 2 (T1R2) subunit to form a distinct sweet taste receptor, which is expressed in the oral cavity and provides input on the caloric and macronutrient content of ingested foods (Carniel Beltrami, Döring, and De Dea Lindner 2018). This receptor recognizes all natural and artificial compounds perceived as sweet by humans (Belloir, Neiers, and Briand 2017).

The receptor cells transmit the information via specific nerve fibers to the brain areas involved in taste processing (Steinert et al. 2011). In the brain, the information deriving from the interaction of the taste receptors with the sweet molecule is processed and interpreted. The generated output at the cerebral level is called "cognitive level". Therefore, physiological and cognitive stimuli are tightly interconnected and both participate in the evaluation of a tasting experience.

The perception of sweetness is determined not only by its intensity, but also by its temporality (persistence). Temporality is defined as how the quality and/or intensity of a perceived food characteristic change over time (McCain, Kaliappan, and Drake 2018). Natural and artificial sweeteners often have an aftertaste and show a temporality different to that of sucrose. For example, aspartame often shows a bitter note at higher concentrations and sweetness lingers much longer compared to sucrose. According to Das and Chakraborty (2015), a possible explanation for the qualitative sweetness differences between natural and artificial sweeteners may be that taste receptor cells respond differently to the group of sweeteners as a result of the often very different chemical structure. Indeed, natural sugars such as

sucrose use the cyclic adenosine monophosphate pathway to elicit membrane depolarization while artificial sweeteners use the inositol triphosphate (IP3) and diacylglycerol pathway for signal transduction.

Every person has its own preference for sweetness, which generally decreases with age (Drewnowski et al. 2012), but also depends on the amount of intake of sweet substances. Mahar and Duizer (2007) showed that women with a high consumption of sweetener (natural or artificial) preferred sweeter orange juice than those persons with a low sweetener intake. Additionally, the perception of a specific sensation can be influenced by simultaneous stimuli of a different sensory modality (cross modal interactions).. For example, the perception of (sweet) taste is influenced by visual cues, auditory cues, smell, the trigeminal system, and touch (Narumi et al. 2011).

7.1 Aroma taste interactions

Taste is primarily perceived on the tongue in contrast to volatile, aroma-active molecules (odorants). The latter can be perceived in two different ways.

- 1) 'Orthonasal' olfaction: Direct action of the aroma active compound from air to odor receptors located in the upper part of the nose. For this type of the term "odor" is used;
- 2) 'Retronasal' olfaction: Indirect action via connection between mouth cavity and nose. In this case, it is called an 'aroma'.

Odors and taste are bound together via associative learning and synthetic attention (i.e. attention to the flavor as a whole in contrast to analytical attention with one stimulus) (Prescott 2015).

Interestingly, during odor characterization, it is very common for panelists to use descriptors that are more associated with taste rather than with odor, such as 'sweet' (Barba et al. 2018). According to Stevenson, Prescott, and Boakes (1995) and Frank and Byram (1988) the perceived sweetness of certain odors could arise from associations with sweet-tasting foods. Odorants can acquire taste-like qualities (e.g., "sweet" odor of vanilla, "salty" odor of sardine) by learned association (Lim, Fujimaru, and Linscott 2014). According to Noble (1996) the perceived intensity of tastes is especially increased in flavored solutions when there is a logical (i.e. congruent) association between them, such as between sweetness and fruitiness, but not for incongruence, like e.g. for ham and sweetness. Odor-induced enhancement of sweetness was observed for different congruent mixtures of sucrose and fruits: with lemon aroma (Frank, van der Klaauw, and Schifferstein 1993); with strawberry aroma (Frank, van der Klaauw, and Schifferstein 1993), with vanilla aroma (Sakai et al. 2001); with green fruity aroma as well with peach aroma (Barba et al. 2018).

In contrast, the perceived quality or intensity of a tastant may be also decreased by the addition of specific odorants (Murphy and Cain 1980; Murphy, Cain, and Bartoshuk 1977). For example, strawberry, caramel, coffee, orange, maracuja and peach odor tended to increase sweetness intensity of sucrose in solution. In contrast, liquorice, damascene, and angelica oil led to a decrease in sweetness intensity. Moreover, the same odor does not always induce an equal effect on sweetness. Lemon seems sometimes to enhance sweet taste intensity, sometimes to suppress it and other times to have no effect. One explanation for these observed differences for the effect of lemon may be influenced by cultural background. For example, the association between lemon and sweet is less frequent in France than in Vietnam where lemon soft drinks are very popular (Valentin, Chrea, and Nguyen 2006).

Most of the studies on taste-odor interactions used aqueous solutions for testing, but also a few were done using dairy products. In whipped cream containing sucrose, sweetness intensity increased when a strawberry flavor was added compared to the product containing only sucrose (Frank and Byram 1988).

The addition of vanilla aroma enhanced sweetness perception in milk (Lavin and Lawless 1998; Wang et al. 2018) and in a cocoa beverage but not in caffeinated milk beverages (Labbe et al. 2006). An increase in vanilla concentration resulted in a higher perceived sweetness intensity also in vanilla flavored dairy dessert (Alcaire et al. 2017). In a study with chocolate flavored milk, the addition of artificial vanilla flavor (0.025%) did not significantly affect citation frequency of the attribute 'sweet' in samples with a 20% reduction in added sugar. In contrast, a high (0.050%) concentration of vanilla flavor increased the sweetness of the chocolate milk with a 40% reduction in sugar. In addition, both concentrations (0.025% or 0.050%) of vanilla flavor increased perceived sweetness intensity in chocolate milk with a 60% sugar reduction (Oliveira et al. 2015).

7.2 Interaction with texture

Many studies have shown that flavor release is linked to the texture of the product, which in turn is dependent on the way the food structure changes during consumption. Flavor release depends not only on the amount of sweet stimulus released in the mouth during food consumption but also on the effects of physicochemical and cognitive interactions (Tournier et al. 2009). Interactions may occur at a neurological level where gustatory and trigeminal inputs converge, or even at a perceptual level where previous dietary experiences could influence taste judgements (Christensen 1980).

The diffusion rate of the stimulus within food, the rheological behavior of the product and the binding of sweet compounds with other food components can potentially impact the flavor release (Arancibia, Costell, and Bayarri 2013). Furthermore, the flavor release during eating could be modulated by mastication and modified by the dilution with saliva (Ruth and Roozen 2000).

Generally, an increase in firmness of a solid food or an increase viscosity of a fluid food results in a decrease of sweetness intensity perceived during eating. Early investigations on threshold values for sweetness of liquids, foams and gels prepared from tomato juice and milk-egg-custard showed that thresholds were lowest in liquids and foams, and highest in gels (Mackey and Valassi 1956). Wagoner et al. (2018) studied the effects of texture on sweetness dose-response profiles of whey protein solutions sweetened with either sucrose, sucralose, stevia, or monk fruit extracts. The more viscous or semisolid samples textures required higher amounts of sweetener to reach iso-sweetness. A possible explanation for this observation is a slowed or partial inhibition of the transport of the sweet stimulus compound both within the food matrix, and also between the food matrix and taste receptors reduces the perception of sweetness intensity (Arancibia, Costell, and Bayarri 2013).

Sweetness perception is also linked to water mobility. With increasing thickener concentration, the volume of free water decreases, leading to a lower water activity and water mobility (Christensen 1980). In consequence, sweetness intensity is decreased, most likely due to a prolonged transport time of the water soluble sweet molecules to the taste receptor cells in the mouth (Christensen 1980). Furthermore, dissociation of free water molecules arranged around the periphery of the sugar molecule produces a high membrane potential across the taste cell, thereby enhancing sweetness perception (Hollowood, Linforth, and Taylor 2002).

Perceived sweetness intensity depends not only on the concentration of the thickening agent but also on the type of the thickening agent. For example, the addition of different types of soluble starch (0.125%-4%) to aqueous sucrose solutions (0.1-1M) resulted in an increase of perceived sweetness

intensity. In contrast, no sweetness enhancement of soluble starch was observed for solutions containing fructose (0.43 M), glucose (0.82 M), sorbitol (0.82 M), aspartame (0.0037 M) Saccharin (0.0042 M) or cyclamate (0.016 M) ((Kanemaru, Harada, and Kasahara 2002).

Tournier et al. (2009) examined texture–taste, texture–aroma and aroma–taste interactions in custard desserts. Taste and aroma did not affect texture perception most likely because the rheological properties of the desserts were not modified. Texture affected the taste intensity but not the aroma intensity, whereas aroma influenced taste perception and vice-versa. As the observed results on cross modal interactions were not always congruent, it was concluded that the examined sensory interactions were specific for both, product and sensory modality.

In gelatin gels, structure modifications at macroscopic or microscopic levels might enhance sweetness intensity due to an inhomogeneous distribution of sucrose (Holm, Wendin, and Hermansson 2009; Mosca et al. 2012; Mosca et al. 2015). Therefore, a possible strategy to increase the sweetness intensity in the context of sugar reduction is to distribute sugar particles in a non-homogenous way in the product.

In terms of rheological properties yogurt is defined as a pseudo-plastic material. Stirred or drinking yogurts show the behavior of a viscoelastic fluid whereas set yogurts belong to the product group of viscoelastic solids (Lee and Lucey 2010). The physical properties of yogurt gels can be explained using a model for casein interactions. There is a balance between attractive forces such as hydrophobic attractions, casein cross-links contributed by calcium phosphate nanoclusters and covalent disulfide cross-links between caseins and denatured whey proteins and repulsive forces e.g. electrostatic or charge repulsions, which are mostly negative at the start of fermentation (Batt and Tortorello 2014). Therefore, changing the protein content is a potential method for modifying yogurt texture and aroma release. Indeed, yogurt enriched with casein had a lower aroma release than yogurt enriched with whey protein (Saint-Eve, Juteau, et al. 2006).

Microscopically it has been shown, that a network of casein micelles linked together in clusters, chains and strands creates pores of variable size in which the aqueous phase is confined (Modler and Kalab

1983). The network formation and textural properties of yogurts can be influenced by technological parameters during production, such as changing the temperature or stirring, whereby the process of stirring results in weaker gels (Lee and Lucey 2010; Lee and Lucey 2004).

Fat reduction in yogurt has been associated with poor texture. Consumers significantly discriminated fat-free/low fat yogurt from the full fat versions with most common verbatim used being "creamy", "sweet" for the full fat versus "watery", "sour" for the fat-free samples (Laguna et al. 2017). According to Sandoval-Castilla et al. (2004) and Torres et al. (2018) the protein network of reduced fat yogurt is less dense, more open, and shows a higher level of serum pores compared to a full fat yogurt. This is a consequence of smaller casein micelles aggregates, probably due to lower number of fat globules acting as linking protein agents.

7.3 Interaction with fat

Besides other functional properties, fats are an excellent solvent for flavor compounds, which show a high hydrophobicity. In sucrose/fat mixtures, in liquids an increasing fat level suppresses sweetness whereas increasing sucrose concentration suppresses the perception of fat in solids (Drewnowski et al. 1989; Hayes and Duffy 2007). Similar results were observed in a model system consisting of skimmed milk, different concentrations of sunflower oil and sweet compounds (including sucrose, aspartame and sucralose) (Wiet et al. 1993). The reduction of fat levels led to a slight increase in perceived sweetness for the three sweetening compounds when tested separately. However, this effect appeared to be non-linear and concentration-dependent, reflecting a complex interaction between fat, type of sweetener, and sweetness level. A study with a dairy-based emulsion found that, despite constant viscosity, an increase in emulsified fat led to an increased sweetness sensitivity. In addition, the fat content also influenced the difference threshold; the higher the concentration of fat in a dairy-based emulsion was, the more a sugar reduction was perceived (Hoppert et al. 2012).

An explanation for this phenomenon could be that fatty acids stimulate taste receptor cells (Mattes 2009). On the other hand, Lynch et al. (1993) showed that a coating in the mouth with coconut oil might suppress sweetness sensation.

In low fat yoghurts, fat content was found to significantly enhance creamy flavor, sweet taste and mouth-feel, but significantly reduce astringency, bitter and sour taste (Folkenberg and Martens 2003). Saint-Eve, Juteau, et al. (2006) concluded that aroma release and perception of sweetness were greater in low-viscosity yogurts with low fat content than in high-viscosity yogurts with high fat content. During phenotypical measurements of oral sensations. Hayes and Duffy (2007) stated that mixtures of fat and sweet compounds may exhibit hedonic synergy; however, the only interaction observed in this study between sucrose and fat was the apparent suppression of sweetness at high levels of fat. In contrast, Tuorila et al. (1993) found that sucrose enhanced perceived fattiness and fat content increased sweetness perception in strawberry yogurts, highlighted the close relationship between perception of sweetness and fattiness in yogurt. Considering the contradictory results found in literature, the role of fat in sweetness perception of yogurt is not clear.

7.4 Interaction with color

Visual stimuli such as for example color strongly influence the expectation about sensory perception during food consumption. For example, consistent color flavor/odor associations have been found between red and pink and strawberry as well as between yellow and orange and lemon (Demattè,

Sanabria, and Spence 2006). A study by Huisman, Bruijnes, and Heylen (2016) showed that round red shapes were associated with sweetness whereas angular green shapes were associated with sourness. However, color and shape visualizations did not influence the taste perception of sweetened and un-sweetened yoghurts.

Other studies showed that the addition of green or yellow color decreased the sweetness in pear nectar or respectively in strawberry and cherry drinks (Pangborn and Hansen 1963; Kostyla, Clydesdale, and McDaniel 1978). In comparison, the addition of red color increased sweetness in both strawberry and cherry drinks (Kostyla, Clydesdale, and McDaniel 1978). In yogurts with a forest berry flavor a higher concentration of red colorant resulted in a higher sensation of sweetness intensity. However this effect was not observed in yogurts with strawberry and orange aroma (Calvo, Salvador, and Fiszman 2001). Moreover, the color of tableware can also influence the sensory perceptions (Huisman, Bruijnes, and Heylen 2016). For example, both white and pink colored yogurt eaten from a white spoon were perceived as sweeter than when eaten from a black spoon (Harrar and Spence 2013). Similarly, red strawberry mousse was rated sweeter when tested from a white plate compared to a black plate (Piqueras-Fiszman et al. 2012).

8 Strategies for sugar reduction

Regarding a healthy and balanced nutrition, sugar reduction is currently a “hot” topic, together with salt and fat reduction, due to the impact on the healthcare system and the promotion of a healthy lifestyle (DLG 2018). Strategies found in literature on sugar reduction in dairy products can be grouped as follows:

- Gradual sugar reduction
- Reduction or total replacement of added sugars by addition of sweetening substitutes
- Sweetness enhancers
- Technological adaptations
 - o use of specific bacterial starter cultures
 - o Use of enzymes, e.g. lactase
 - o food micro-layering with sugar
 - o spatial distribution of sugar in the matrix and sugar encapsulation
- take advantage of sensory interactions
- Simultaneous application of different above-mentioned strategies

The main and most promising strategies are discussed below, also considering their strengths and limitations.

8.1 Gradual sugar reduction

For products already on the market, one of the most promising strategies for a sugar reduction in terms of effectiveness, potential, and immediate results is a gradual decrease of sugar concentration over time (Ma et al. 2016). The idea of this strategy is based primarily on the hypothesis that there will be an overall downward shift in sweetness preference following a period of exposure to sugar-reduced products. The two main strategies for a gradual reduction in sugar content are

- imperceptible reduction of the sugar content = "silent" reduction

The consumer does not notice the reduced sweetness intensity associated with the reduction.

- perceptible reduction of the sugar content

The consumer perceives the lower sweetness intensity associated with the reduction. The reduction in sugar content is deliberately high and is often implemented and advertised in new product lines. The change in the sensory profile of the product caused by the sugar reduction does not always lead to a reduction in the acceptance of the product.

8.1.1 Silent reduction

The most common method to find the concentration steps by which sucrose can be reduced without resulting in a perceptible sweetness difference is the determination of the difference threshold. The difference threshold or "just noticeable difference" (JND) is defined as the smallest difference in sugar concentration that causes a difference in the perception of sweetness intensity for at least 50% or 75% of the individuals, depending on the stringency of the test (Lawless and Heymann 2010; Lima, Ares, and Deliza 2018). The difference threshold, as any other sensory threshold, depends strongly on the food matrix and therefore, has to be determined individually for each product.

JND values for sweetness for different food and beverages reported in the literature are limited. Determined values were: 6.7% in chocolate flavored milk (Oliveira et al., 2016), 6.21% of the added sugar content in grape nectar (Lima, Ares, and Deliza 2018), 8.5% in orange nectar (Pineli et al. 2016) and 7.0–11.0% in pound cakes (Chang and Chiou 2006). Starting at five different initial sugar concentrations levels difference thresholds between 4.2 and 8.14% of the added sugar concentration were determined in three different fruit nectars (passion fruit, range/passionfruit and orange/pomegranate) (Oliveira et al. 2018).

These results support the theory that JND's follow Weber's law. This psychophysical law states that the difference threshold between two stimuli is not an absolute value, but depends on the level of the initial concentration of the stimulus (reference of Weber). It is also suggested that difference thresholds are a

constant proportion of the stimulus intensity (Lawless and Heymann 2010). Stevens's Law, was found to better match the stimulus-response curves for certain sensory phenomena, such as smell and taste, as the stimulus intensity in the equation is additionally raised to a higher power (Nutter 2010).

8.1.2 Perceptible reduction of sugar content

Research on chocolate milk with Uruguayan consumers showed that sequential sugar reductions can be set at 6.7% without affecting consumers' sensory and hedonic perception. A further sugar reduction of up to 29% did not result in different overall liking (Oliveira et al. 2016). A previous study with the same product showed that a 20% reduction of added sugar resulted in changes of sweetness intensity, which was perceived by both, trained assessors and consumers. However, product liking was not significantly affected by sugar reduction up to 40% of the initial concentration of 9% added sucrose (Oliveira et al. 2015). The evaluation of acceptability of chocolate milk showed that a sugar reduction up to 30% (from 205mmol/L sucrose) was accepted by both, young adults and children (Li et al. 2015).

Results of a study with mocha and strawberry yogurts suggested that Swiss consumers showed a preference for sugar-reduced yogurt compared to the sucrose level usually found in commercially available yogurts (Chollet et al. 2013). Similarly, in a study with Moroccan consumers evaluating yoghurts from the market and sugar reduced yogurts (20-30% reduction of added sugar) the latter showed the highest acceptance (Benkirane et al. 2017).

Brazilian consumers testing sucrose reduced orange nectars (juices with added sugar) showed a clear difference in sweetness liking. While nectars with 5.5% added sucrose were ideally sweet for one group, samples with 10.5% added sugars showed an ideal sweetness for the second group (Pineli et al. 2016). The average ideal sweetness among all consumers was determined for samples containing 7.3% sucrose. The rejection threshold i.e. the point where preference was significantly lower than for the reference was found at a concentration of 7.2% (Pineli et al. 2016).

In sugar reduced grape nectars reduction steps bigger than JND of 6.21% resulted in distinctly perceptible changes in the sensory characteristics, but had only little effect on average overall liking scores (Lima, Ares, and Deliza 2018). Children had more problems to recognize changes in the sensory profile but were more sensitive towards liking compared to adults.

Similar results were observed in a test with 50 consumers evaluating different fruit nectars. No significant differences in overall liking were detected for products with a 20% sugar reduction. However, consumers showed a broad range of different hedonic reactions towards sugar reduced juices

(Oliveira et al. 2018). In conclusion, results found in literature suggest that a high percentage of consumers accepts a sugar reduction bigger than the difference threshold without changing the hedonic judgement at least for different fruit juices and fruit nectars.

8.1.3 Time interval between reduction steps

There is almost no information in the literature about the time interval by which the next step for a sugar reduction could be set. However, experiences with salt reduction could probably be partially transferred to sugar reduction.

According to Wise et al. (2016), when individuals adopt a sodium-lowered diet, they perceive a given concentration of sodium to be more salty than it was before starting their diet or preferred products with reduced salt content over time. Therefore, gradual salt reduction might lead to a decrease in preferred saltiness intensity and, consequently, to a lower sodium intake. This hypothesis has been confirmed in a study by Methven, Langrenney, and Prescott (2012), where individuals receiving a soup with no added salt showed an increase in liking after only three times of exposure.

In a study on salt reduction in white bread, a 25% reduction in the sodium content could be achieved with reduction steps of 5% for six consecutive weeks. The consumer acceptance was not affected as no differences in the scores for flavor or liking of the bread were observed (Girgis et al. 2003).

In a study by Wise et al. (2016) subjects rated intensity and pleasantness of vanilla puddings and raspberry beverages with various sucrose concentrations during five consecutive months. The low-sugar subjects rated both low and high sucrose concentrations in puddings as 40% sweeter than the corresponding control group. A less pronounced effect on rated sweetness was observed for the beverages. Rated pleasantness was not affected for neither of the two products.

In another study, a group of 20 adults had to avoid all products with added sugars and sweeteners for two weeks (Bartolotto 2015). After this period, 95% of participants found that sweetened food and beverages tasted sweeter than before the abandonment of sweet products. Additionally, 86.6% of participants stopped their craving for sugar after six days.

In contrast, a four-week exposure to sugar-reduced biscuit (28% sugar reduction) did not affect the participants' liking of the original biscuit but did increase liking for less reduced variants (Biguzzi, Schlich, and Lange 2014). In a second study, a stepwise exposure to sugar-reduced biscuits did not affect liking either (Biguzzi, Lange, and Schlich 2015). Two sequential sugar reduction steps of 6.66% each in one

year were recommended for chocolate milk (Oliveira et al. 2016). In summary, in all the mentioned studies the sugar reduction intervals were similar or shorter than 6 months.

Hutchings, Low, and Keast (2019) argued that sweetness intensity of products has to be maintained, if sweetness is the most salient sensation that needs to be modified, in order to guarantee consumer acceptance.

8.2 Replacement of sugars

The majority of studies on sucrose reduction belongs to the strategy of replacement of sugar by other ingredients that show sweetening and/or textural characteristics together with a lower energy content compared to sucrose. Table 2 shows an overview of common sweet-tasting compounds (Edwards et al. 2016).

Table 2: Overview of common nutritive and non-nutritive sweet-tasting compounds and their relative sweetness, glycemic index and caloric value (Edwards et al. 2016).

SWEETENER	RELATIVE SWEETNESS	GLYCAEMIC INDEX	ENERGY (kcal/g)
Nutritive Sweeteners			
Sugars (NS)			
Monosaccharides			
Glucose	50 ^b	100 ^b	4 ^b
Fructose	150 - 180 ^b	19 - 23 ^b	4 ^b
Galactose	26 ^c	23 ^c	4 ^b
Disaccharides			
Maltose	40 ^b	105 ^b	4 ^b
Sucrose	100 ^b	61 - 65 ^b	4 ^b
Lactose	20 - 40 ^b	46 ^b	4 ^b
Bulk Sweeteners (NS)			
Erythritol	60 - 80 ^a	0 ^b	0.2 ^b
Isomalt	45 - 65 ^b	2 ^b	2.0 ^b
Lactitol	35 - 40 ^b	6 ^b	2.4 ^b
Maltitol	50 - 90 ^b	35 - 52 ^b	3.0 ^b
Mannitol	50 - 72 ^b	0 ^b	1.6 ^b
Sorbitol	50 - 100 ^a	9 ^b	2.6 ^b
Xylitol	100 ^b	7 - 13 ^b	3.0 ^b
Tagatose	92 ^b	0 ^b	1.5 ^b
Non-nutritive Sweeteners			
Acesulfame-K	20 000 ^a	0	0
Aspartame	18 000 - 20 000 ^a	0	4
Cyclamate	3 000 ^a	0	0
Neohesperidin DC	190 000 ^a	0	0
Saccharin	30 000 - 50 000 ^a	0	0
Sucralose	60 000 ^a	0	0
Thaumatococin	200 000 - 300 000 ^a	0	0
Steviol glycosides	1 000-1 500 ^b	0	0

Data from ^a(Mortensen 2006), ^b (Chattopadhyay, Raychaudhuri, and Chakraborty 2014), and ^c (Coultate 2016)

Generally, sugar replacers are classified in different groups based on their chemical/physical characteristics, suitability for different product categories (liquid or solid) and sweetening power. In this report the classification according to (Lê, Robin, and Roger 2016) is used.

Table 3: Different classes of sugar replacers and their characteristics (Lê, Robin, and Roger 2016).

Sweetness replacers	Bulking replacers
High Intensity Sweeteners (HIS) <ul style="list-style-type: none"> - High sweetening power - Very low to low caloric density - Most suitable for liquid products 	Sugar alcohols <ul style="list-style-type: none"> - Low sweetening power - Low caloric density - Similar physicochemical properties to sucrose - May cause laxative effects depending on daily dose
Taste enhancers <ul style="list-style-type: none"> - No sweetening power - Very low caloric density - Most suitable for liquid and semi-solid products - Declared as additives 	Alternative/rare sugars <ul style="list-style-type: none"> - Low to moderate sweetening power - Low to neutral caloric density - Similar physicochemical properties to sucrose
Flavors with modifying properties <ul style="list-style-type: none"> - No sweetening power - Very low caloric density - Most suitable for liquid and semi-solid products 	Dietary fibers <ul style="list-style-type: none"> - No sweetening power - Low caloric density - Most suitable for semi-liquid and solid products - Unfavourable taste and texture profile in some cases
	Malto-Oligosaccharide <ul style="list-style-type: none"> - Low to neutral sweetening power - Neutral caloric density

According to Lê, Robin, and Roger (2016) sugar replacers are classified into two main groups, namely sweetness replacers and bulking replacers (Table 3). Compounds belonging to the group of sweetness replacers are used for a compensation of the perceived decrease in sweetness intensity, while bulking replacers mainly compensate for the lack of bulk and texture.

The majority of sweetness replacers is characterized by a much sweeter taste intensity than mono- or disaccharides. Whereas natural sweetness replacers show a sweetness potency range of 0.1 to 450, artificial sweeteners show a sweetness potency up to 20'000 times higher than sucrose (McCain, Kaliappan, and Drake 2018). Taste enhancers also belong to the group of sweetness replacers. These substances do not elicit a sweet taste themselves, but in combination with specific compounds, they are able to enhance perceived sweetness. The group of "flavors with modifying properties" encompasses

substances that are able to modify the flavor profile of a food product and, as a side effect modify the sweetness perception.

Sugar alcohols obtained by hydrogenation of sugars are classified as bulking replacers. This bulking replacer group also contains alternative sugars that represent isomers of the common sugars, dietary fibers of plant origins that cannot be completely digested by humans, as well as malto-oligosaccharides, which are derived from glucose monomers. Besides their desired bulking properties, the majority of bulking replacers also show sweet characteristics. However, with few exceptions, the sweetness intensity evoked by bulking agents is only moderate. Therefore, they usually have to be used in combination with other sweet tasting substances.

Sugar replacers can either modulate the quality and/or also the temporal perception of sweetness profile. A major drawback of several sugar replacers is their pronounced 'off' flavor, which often limits their use in food products (Du Bois and Prakash 2012).

Although low-calorie sweeteners used also in Europe have been approved as safe and have been subjected to extensive scientific testing, confusion about their impact on health remains in the consumer's mind (Buttriss 2017). Thus, not surprisingly consumers tend to prefer a "naturally sweetened" product over an "artificially sweetened" equivalent product (McCain, Kaliappan, and Drake 2018). Nevertheless, in blind testing chocolate flavored dairy desserts sweetened with artificial sweeteners got higher scores compared to dairy products containing natural sweeteners (Morais et al, 2014).

Another disadvantage of sugar replacers is that they are artificial and have to be declared on the ingredient list, which is against the observed trend that consumers desire "all natural" products (McCain, Kaliappan, and Drake 2018). Additionally, there are still some legislation gaps and differences between countries. For example cyclamate and neohesperidine dihydrochalcone are allowed in the EU and not in the US, while D-tagatose is not approved as a sweetener in the EU and cannot be used in these countries in food production (Carocho, Morales, and Ferreira 2017).

8.2.1 Sweetness replacers

8.2.1.1 High-intensity sweeteners (HIS)

High-Intensity sweeteners (HIS) are compounds of natural and artificial origin with a strong sweetening power. They are mainly used to add sweetness to energy-reduced products or products with no added sugars. Well known high-intensity sweeteners are aspartame, acesulfame K, rebaudioside A, saccharin, sucralose and neotame (Table 4).

However, their application is limited by the different sensory temporality compared to sucrose. Temporality is defined as how perception of sensory characteristics of a food product change over time. None of the nonnutritive sweeteners currently on the market is able to perfectly match the temporality of sucrose (Lawless and Heymann 2010; Morais et al. 2014). Moreover, nonnutritive sweeteners can also elicit additional sensations including metallic mouthfeel and bitter taste. In consequence, food producers try to mask these unwanted sensory qualities of nonnutritive sweeteners.

Table 4: High Potency sweeteners, their chemical/sensory properties, costs and regulation (Aidoo et al. 2013).

	Aspartame	Acesulfame-K	Rebaudioside A	Saccharin	Sucralose	Neotame
Sweetness potency (times that of sucrose)	180–200	130–250	200–300	300–500	500–700	8000–13,000
Taste/Profile	Slow onset, lingering sweetness Clean sweetness with little to no aftertaste	Quick onset with no significant lingering sweetness Can have a bitter aftertaste	Moderate to quick onset with little to no lingering sweetness Potential for bitter or black licorice aftertaste	Quick onset with no significant lingering sweetness Potential for metallic, bitter aftertaste	Clean sweetness with slow onset and lingering sweetness	Slow onset, lingering sweetness Clean sweetness with little to no aftertaste
Stability	Limited stability at elevated temperature and low pH	Good stability at elevated temperatures and low pH	Good stability at elevated temperatures and low pH	Good stability at elevated temperatures and low pH	Good stability at elevated temperatures and low pH	Limited stability at elevated temperature and low pH
Blending options	Good synergy with acesulfame-K and saccharin	Good synergy with aspartame and sucralose	N/A	Good synergy with aspartame and sucralose	Good synergy with acesulfame-K and saccharin	Good synergy with acesulfame-K and saccharin
Advantages	Widely used, sweetness profile and cost-effectiveness	Stability and synergies with other HPSs	“Natural” status and stability	Cost-effectiveness and stability	Sweetness profile, branding and stability	Sweetness profile and cost-effectiveness
Regulatory ^a status	Food additive ADI 50 mg/kg of body weight/d	Food additive ADI 15 mg/kg of body weight/d	–	Permitted for use under an interim regulation	Food additive ADI 5 mg/kg/d	Food additive ADI 18 mg/p/d

^a ADI values listed here are those established by the US Food and Drug Administration (expressed in milligrams per kilogram of body weight per day). Neotame is however expressed in terms of milligrams per person per day (mg/p/d)

Addition of HIS to yogurt has been widely investigated. In an early study by Hyvönen and Slotte (1983) saccharin was used as a replacer of sucrose in yogurt. However, the bitter aftertaste of saccharin made it necessary to mix it with xylitol, a sugar alcohol, to cover the negative qualitative aspects of saccharin. The study by Keating and White (1989) tested a wide palette of sweeteners such as aspartame, calcium saccharin, sodium saccharin, sorbitol, high fructose corn syrup, high fructose corn syrup plus monoammonium glycyrrhizinate, sucrose plus monoammonium glycyrrhizinate (MAG), acesulfame-K. and dihydrochalcone in plain and flavored (strawberry, cherry) yogurts. The expert panel (n=2) preferred the yoghurts with sucrose for plain as well as flavored yoghurts. The lowest score was given to the yoghurt sweetened with dihydrochalcone. Samples sweetened with sorbitol were closest to the samples with added sucrose. In contrast, the strawberry sample sweetened with sorbitol was among the least accepted products when evaluated by consumers (n=60) who clearly preferred the yoghurt sweetened with sucrose followed by the yoghurt containing aspartame

There is also a number of less well known HIS sweeteners that have been tested in yogurt. A study on the possible application of neohesperidine DC added to milk products showed that this sweetener was stable throughout pasteurization and fermentation processes (Montijano, Thomas-Barberan, and Borrego 1995).

Currently, for sugar reduction in dairy products. artificial sweeteners are still more commonly used than natural non-nutritive sweeteners. However, artificial sweeteners are becoming less popular due to the stigma of the term “artificial” and the changing landscape of food (McCain, Kaliappan, and Drake 2018). Among the alternative non-nutritive sweeteners natural extracts that show a high sweetness potency, such as stevia or monk fruit have gained interest.

Several authors evaluated the potential use of stevia in yoghurts (Fayaz et al. 2018; Guggisberg, Piccinali, and Schreier 2011; Kalicka et al. 2017; Narayanan et al. 2014; Rodriguez Furlán and Campderrós 2017).

Most of these studies showed that consumers preferred products for which stevia was used in combination with another sweetener compared to samples with stevia as the only sweetener. However, the application is limited by the different sensory temporality compared to sucrose. Moreover, nonnutritive sweeteners can also elicit additional sensations including metallic mouthfeel and bitter taste. In consequence, food producers try to mask these unwanted sensory qualities of nonnutritive sweeteners.

The influence of different combinations of stevia (0-0.04%) and oligofructosaccharide (0-6%) was tested in low fat (0.1%) and full fat (3.5%) plain set yoghurt and compared to a yoghurt containing 8% sucrose (Guggisberg, Piccinali, and Schreier 2011). All tested stevia/oligofructosaccharide samples resulted in lower perceived sweetness intensity compared to the yoghurt sweetened with sucrose. A negative off-flavor described as cardboard, metallic and artificial was perceived for the yogurts with stevia. The sample sweetened only with stevia (0.04%) was also judged as significantly more bitter compared to the other samples. The combination of 6% oligofructosaccharide with 0.025% stevia showed the sensory profile closest to the sample sweetened with sucrose (Guggisberg, Piccinali, and Schreier 2011).

Narayanan et al. (2014) tested different stevia sweeteners and aspartame in low-fat vanilla flavored yogurts. Sweetness and sourness were influenced by the stevia concentrations. Consumers did not like yogurts sweetened with only the stevia sweetener or aspartame.

Even so yogurt sweetened with stevia and yoghurt samples produced with sucrose were perceived as equi-sweet in intensity, the quality of sweetness of the yoghurts with added stevia was judged as more persistent and considered less natural (Kalicka et al. 2017).

Similarly, a study by Reis et al. (2011) evaluating equi-sweetness of strawberry-flavored yogurt with different sweeteners and/or their combinations (aspartame, acesulfame-K, cyclamate, saccharin, stevia and sucralose) to yogurt sweetened with 11.5% w/w sucrose found that stevia used as the only sweetener altered the color and flavor of the strawberry-flavored yogurt. These authors recommended the addition of a combination of stevia with acesulfame-K to sweeten strawberry-flavored yogurt (Reis et al. 2011).

Following the trend towards natural ingredients, monk fruit in combination with stevia has been tested in different dairy products such as vanilla whey protein drink with the goal to formulate natural sweetener blends that closely matched the temporality of a corresponding sucrose-sweetened drink (Parker, Lopetcharat, and Drake 2018). Priming statements about the sweetener used in each beverage were displayed before taste sessions. Two distinct consumer clusters were identified: i) label-conscious consumers who preferred beverages sweetened with natural blends when primed and ii) the flavor-driven segment of consumers who conceptually preferred naturally sweetened beverages but actually preferred sucralose-sweetened beverages when primed (Parker, Lopetcharat, and Drake 2018).

A similar result was observed for chocolate milk (Li, Lopetcharat, and Drake 2015). Chocolate milk sweetened solely by nonnutritive sweeteners (stevia leaf and monk fruit) were less accepted compared

to a sucrose control (young adults, aged 19 to 35, n=131). The presentation of chocolate milk label information influenced parental acceptance. Traditional parents preferred sucrose-sweetened chocolate milk whereas label-conscious parents preferred chocolate milk with natural nonnutritive sweeteners (Li, Lopetcharat, and Drake 2015).

Of the four major steviol glycosides, Rebaudioside A was considered the least astringent and least bitter with a less persistent aftertaste. It was judged to have the most favorable sensory attributes (Das and Chakraborty 2015). Both, stevioside and rebaudioside A, can be used as synergistic ingredients in mixtures with other high-potency sweeteners (Das and Chakraborty 2015). Both, stevioside and rebaudioside A, can be used as synergistic ingredients in mixtures with other high-potency sweeteners (Das and Chakraborty 2015).

Although widely discussed, there is currently no distinct evidence that HIS have detrimental effects on health (Lê, Robin, and Roger 2016). Indeed, acesulfame K, aspartame, advantame, neotame, saccharin, sucralose, stevioside and rebaudioside A (both sweet extracts of the *S. Rebaudiana* Bertoni plant) as well as monk fruit (Luo Han Guo) were recognized as safe (GRAS) by the US Food and Drug Administration (FDA) (Sylvetsky and Rother 2016). In Switzerland, stevioglycosides are allowed whereas monk fruit is not allowed as food additives (FSVO 2019a).

8.2.1.2 Taste enhancers

Sweetness enhancers are non-caloric molecules capable of augmenting or amplifying the perceived sweetness intensity of sucrose. They are considered as food additives and their primary effect is the enhancement of the sweetness. Since these compounds are added in extremely low quantities, they neither contribute to the caloric content of a food nor have they an unwanted impact on the manufacturing process (Lê, Robin, and Roger 2016).

So called positive allosteric modulators (PAMs) are compounds that enhance the activity of the sweet taste receptor through binding at an allosteric site without showing a sweet taste itself (Du Bois and Prakash 2012). In consequence, when using such PAMs, the sucrose concentration in food or beverage could be reduced while maintaining the desired sweet intensity level (Servant et al. 2011). A first set of PAMs (SE-2, SE-3, and SE-4) was tested for the sweet taste receptors in cell-based assays and simple taste tests in which the sweetener was dissolved in water. SE-2 is a selective enhancer for sucralose, whereas SE-3 enhances the sweet taste of both sucralose and sucrose (Zhang et al. 2010).

More examples of PAMs potentially enhancing the sweetness intensities of common carbohydrate sweeteners have been suggested by (Du Bois and Prakash 2012). According to these authors, future challenges will be the search for natural PAM's showing a higher enhancing potential (i.e. 10- to 20-fold enhancers).

8.2.1.3 Flavors with modifying properties (FMP)

In the United States, in order to qualify as a flavoring substance with flavor modifying properties (FMP), a prospective flavoring substance must be shown not to possess an inherent taste (e.g. sweetness) and must modify flavor attributes at the desired level (Harman and Hallagan 2013). The term "flavoring with modifying properties" (FMP) has not been defined in the European regulation but can be interpreted as those categories of flavoring compounds which are able to modify odor and / or taste of the food (EFFA 2015).

FMP can increase, decrease or change the perception of individual sensorial flavor characteristics.

However, in contrast to sweet taste enhancers, sweetness modification is considered a secondary effect of FMP's. In general, FMP have no or only few calories and extremely low quantities are sufficient to induce an effect (Lê, Robin, and Roger 2016).

FMP that belong to the group of positive allosteric modulators (PAMs) of the sweet human taste receptors and have the ability to enhance the receptor activity and thus, sweetness perception. Two of these PAMs (S6973 and S617) have been shown to function in food and beverage products with reduced quantities of sweeteners to restore the initial level of sweetness intensity as well as to modify other flavor attributes, such as juicy, lemony, green notes, licorice, and clove notes. These two PAMs were reviewed by the FEMA and classified as GRAS for the use as flavor ingredient. (Arthur et al. 2015). However, long-term effects of these compounds are not yet known. There is also data available on the potential use of these compounds for sugar reduction in dairy products such as yogurts.

8.2.1.4 *Addition of proteins for flavor enhancement*

Dairy foods usually contain notable concentrations of proteins usually divided in the hydrophobic fraction of caseins and the water-soluble lactalbumins and lactoglobulins found in the whey. Currently, there is a reformulation 'wave' of various food products, including yogurt towards an increased protein content (Jørgensen et al. 2019).

Moreover, yogurts fortified with proteins may have a positive influence on consumer acceptance and possibly also on the perceived sweetness. In fact, it was shown that whey protein-yogurts obtain higher flavor and overall liking scores than non-fortified yogurts (Berber, Gonzalez-Quijano, and Alvarez 2015). The addition of whey proteins in yogurt production is a well-established method to increase the total solid content and to improve consistency, texture and creaminess of the product. Whey proteins also have important structural properties such as emulsifying, water/fat aggregation, foaming, thickening, and gelling properties (Donato and Guyomarc'h 2009). Moreover, whey protein powder stimulates bacterial growth and the production of acetaldehyde, one of the main aroma-active compounds of yogurt. In general, an addition of whey powder to produce protein enriched yogurts results in a lower acidification rate during storage. Sweet whey powder containing up to 70% lactose of the total solids can also contribute to the perceived sweetness of dairy foods.

Whey protein concentrates and isolates vary in their content of β -lactoglobulin, degree of lactosylation, mineral content as well as the extent of denaturation. These differences might be responsible for the contradicting results found in the literature on interactions between whey proteins, texture and flavor of yogurt. For example, a high native to denatured whey protein ratio improved the creaminess and viscosity as well as creamy flavor of low fat yogurt (Torres et al. 2011).

The type as well as the concentration of proteins influence the aroma release in yogurts (Saint-Eve, Lévy, et al. 2006). Caseinate enriched yogurts showed a higher complex viscosity compared to products enriched with whey proteins and release fewer aroma compounds (Saint-Eve, Juteau, et al. 2006).

Stirred non-fat yogurts fortified with whey protein isolate (WPI) showed lower diacetyl release compared to samples fortified with skimmed milk powder or caseinate (Chua 2013). No significant differences in acetaldehyde or butyric acid release were found when caseinate to WPI ratios were altered in non-fat stirred yogurts. However, non-fat stirred yogurts fortified with WPI appeared to show a higher reduction of acetaldehyde release compared to yoghurt samples fortified with sodium caseinate (Chua 2013).

According to Isleten and Karagul-Yuceer (2006) yogurt containing WPI had the lowest value for the attribute "fermented flavor" compared to low fat yogurt with skimmed milk powder (control) and yogurt

with sodium caseinate. In general, yogurts fortified with sodium caseinate displayed favorable physical and sensory properties compared to control or WPI-fortified yogurts, and had similar flavor and acceptability as the control products (Isleten and Karagul-Yuceer 2006).

Changing the ratio of casein to whey protein concentrate (CN:WP) has been shown to influence texture, taste profiles and aroma release. Strawberry yoghurts with a high CN:WP ratio were perceived as less fruity and less flavorful compared to samples with a low CN:WP ratio (Saint-Eve, Martin, et al. 2006). A decrease in CN:WP ratio also resulted in a reduced sourness, astringency and aroma perception of stirred yoghurt (Saint-Eve, Lévy, et al. 2006).

In contrast, yogurts with lower ratio of CN:WP (4, 4.5 and 5%) were firmer and stiffer than those with higher CN:WP although alteration of CN:WP did not significantly alter volatile aroma compound release from yogurts (Tomaschunas et al. 2012).

The choice of the milk and whey protein concentrate seems to be important to avoid acidic, bitter, and astringent mouthfeel as well as burnt/beefy flavors caused by sulfurous compounds (Jørgensen et al. 2019).

The use of whey protein seems to be an interesting option for sugar-reduced yogurt to increase the aroma and sweetness. A crucial aspect seems to be the production method of the whey protein to maintain a high ratio of non-denaturated protein. In addition, the use of whey proteins in dairy products has been widely investigated and is a well-regulated technique, which is considered safe for human health.

Sweet proteins (Table 5) might play an important role in food and taste research in the future, but their safety for human consumption has not been yet proven for many compounds. Thaumatin has been approved for use in many countries and by the FDA as both a flavor enhancer and high-intensity sweetener. In Japan, monellin is also approved as a food additive and sweetener. Of all the currently known sweet proteins, brazzein seems to be the most promising of this compound range, because it tastes like sucrose and maintains its properties over a wide range of temperatures and various pH levels.

Table 5: Comparison of thaumatin, monellin, mabinlin, pentadin, brazzein, curculin and miraculin characteristics (Kant 2005).

	Thaumatococcus danielli Benth	Dioscoreophyllum cumminsii Diels	Capparis masakai Levl	Pentadiplandra brazzeana Baillon	Pentadiplandra brazzeana Baillon	Curculigo latifolia	Richadella dulcifica
Source	Thaumatococcus danielli Benth	Dioscoreophyllum cumminsii Diels	Capparis masakai Levl	Pentadiplandra brazzeana Baillon	Pentadiplandra brazzeana Baillon	Curculigo latifolia	Richadella dulcifica
Geographic distribution	West Africa	West Africa	China	West Africa	West Africa	Malaysia	West Africa
Variants	I, II, a, b, c ^a	-	I, II- a, III, IV ^a	-	-	-	-
Sweetness factor (weight basis)	3000	3000	100	500	2000	550	-
Molecular mass (active form, kDa)	22.2	10.7	12.4	12.0 ^b	6.5	24.9	98.4
Amino acids	207	45 (A chain) 50 (B chain)	33 (A chain) 72 (B chain)	?	54	114	191
Active form	Monomer	Dimer (A + B)	Dimer (A + B)	?	Monomer	Dimer (A + A)	Tetramer (A+A+A+A)

Source: Adapted from Kurihara (1994). ^aAt least five different forms of thaumatin (Lee et al., 1988) and four different forms of mabinlin (Nirasawa et al., 1994) have been identified. ^bA chromatographic fraction containing a 12-kDa protein was sweet. This same fraction, when subjected to electrophoresis under non-reducing conditions showed bands in the region between 22 and 41 kDa, suggesting the presence of subunits.

The potential application of natural sweet proteins in yogurt was studied by Miele et al. (2017), who showed that the addition of monellin to yoghurt did not change rheological properties or the fermentation process, but the compound lost its sweetening power when it was added before fermentation. The comparison of the sweetness profile of monellin in set and stirred yogurt with the profile of other natural sweeteners, sucrose, xylitol, and sorbitol showed similar temporal patterns for all tested sweetening agents with the exception of the temporal end phase of sweetness perception. In particular, in stirred yogurt monellin resulted in a lingering sweet aftertaste. In set yogurt, the iso-sweet concentrations of xylitol and sorbitol, but not of monellin were determined, whereas, in stirred yogurts, this concentration could be determined for all the sweeteners (Miele et al. 2019).

Addition of thaumatin was tested in a study with chocolate milk (with 60% sugar reduction) (Oliveira, Deslandes, and Santos 2015). Thaumatin concentrations between 5 and 10 ppm resulted in a significant increase in sweetness perception only when added at the highest of the tested concentrations (10 ppm). This result suggests that thaumatin might not be a feasible option for increasing sweetness perception in chocolate-flavored milks (Oliveira et al. 2015).

Miraculin, a glycoprotein found in miracle fruit (*Synsepalum dulcificum*) can turn a sour taste into a sweet one. Miraculin has been shown to enhance the sweetness of a low sugar dessert (Wong and Kern 2011) and has been used as a sugar replacer in sour beverages (Igarashi et al. 2013; Rodrigues et al. 2016). The few studies that have used sweet proteins in dairy products suggest that the use of sweet protein in sugar-reduced yogurt could be an interesting option. However, the effect of these proteins on the

physical and sensory characteristics of the final product must be further evaluated, since there is not much data available on the use of these compounds in food products.

8.2.2 Bulking replacers

8.2.2.1 Sugar alcohols

Sugar alcohols, also called polyols or polyhydric alcohols, are a group of carbohydrates obtained by substitution of an aldehyde with a hydroxyl group. According to chemical structure sugar alcohols can be grouped in hydrogenated monosaccharides (sorbitol, mannitol, xylitol, erythritol), hydrogenated disaccharides (isomalt, maltitol, lactitol) and mixtures of hydrogenated mono-di- and/or oligosaccharides (hydrogenated starch hydrolysates). Small quantities of polyols are naturally present in fruits, vegetables, and mushrooms. Characteristics of different sugar alcohols are described in table 6.

Table 6: Characteristics of sugar alcohols (Aidoo et al. 2013).

Polyol	Properties
Sorbitol	Derived from glucose; 60% as sweet as sucrose. Good solubility – 70% at 20 °C. Melting point – 97.2 °C. Very hygroscopic and has a cooling effect in crystal form only.
Xylitol	Derived from xylose. Equal in sweetness to sucrose. It has a solubility of 63% with low melting point of 94 °C. Less laxative and less hygroscopic.
Isomalt	Derived from sucrose; about 40% as sweet as sucrose. Has solubility of 25% at 20 °C which increases with temperature. Melting point between 145 and 150 °C. Not hygroscopic, forms agglomerates with high residual moisture. Less viscous thereby decreasing the viscosity of other polyols.
Mannitol	Derived from mannose; about 70% as sweet as sucrose. It crystallizes out because of the poor solubility – 18% at 20 °C. Melting point between 165 and 169 °C. Not hygroscopic but has the highest laxative effect.
Maltitol	Derived from glucose syrup; 95% as sweet as sucrose. Has a solubility of 62% at 20 °C with a melting point lying between 130 and 135 °C. Very hygroscopic.
Lactitol	Derived from lactose; about 40% sweet as sucrose and exists in two forms – monohydrate and anhydrous with melting points of 75 °C and 120 °C respectively. Less hygroscopic than sorbitol or xylitol.
Erythritol	Derived from fermentation of glucose and sucrose by <i>Trichosporonoides megachiliensis</i> ; about 60–80% sweet as sucrose. Has humectant and bulking properties and produces laxative effect upon high consumption.

Sugar alcohols are used to add bulk and texture to food products and often elicit a sweet taste. However, the sweetening power of these compounds is lower compared to monosaccharides. Thus, sugar alcohols are often used in combination with other sweeteners to achieve the desired level of sweetness and flavor (Grembecka 2015). Polyols can also improve the stability and shelf life of a product by influencing its moisture retention and crystallization. Moreover, they are well-established ingredients as prebiotics in food, beverage, confectionery, including dairy products. Unlike other sugar replacers, sugar alcohols carry almost as many calories as sucrose but they do not increase blood glucose levels. They also help to protect teeth from damage, since they are not converted to acids by oral bacteria. However, some sugar alcohols like xylitol or sorbitol are only partially digested and absorbed in the small intestine. So, when they reach the large intestine they can be metabolized by gut bacteria, which result in undesirable laxative effects. Sugar alcohols are labelled as safe food additives with an E number (Grembecka 2015). Despite their favorable properties, which make sugar alcohols suitable for an application in yogurt, only a few studies with polyols in yogurt were found. Hyvönen and Slotte (1983) compared the sensory profile, as well as acidity, viscosity and microbiological quality of sorbitol- and xylitol-containing yogurt and sucrose-containing yogurt. Xylitol was found to be a suitable pre-incubation yogurt sweetener, while sorbitol showed an inhibition effect of bacterial growth of yogurt. In a recent study by (Miele et al. 2019), the iso-sweet concentrations of xylitol and sorbitol were determined in set and stirred yogurt. These two compounds showed similar temporal sweetness characteristics as sucrose. Xylitol was also tested in dadih, a dairy-based dessert made of milk, water, sugar and agar. The presence of xylitol contributed to a softer texture compared to a product that was made only from sucrose (Mohd Thani et al. 2014). For flavored yogurt drinks the addition of maltitol and/or maltitol syrup to replace the sugar solids was recommended to improve texture and sweetness profile (O'Donnell and Kearsley 2012). Considering the low glycemic effects, polyols constitute a valuable choice as sugar replacer when administered within the recommended range. However, the overall polyol content of the product should remain below 20 g per serving to avoid laxative effects.

8.2.2.2 *Rare or alternative sugars*

The international society of rare sugars (ISRS) defines “rare sugar” as “monosaccharides and their derivatives that are rare in nature”. The group of rare or alternative sugars comprises isomers of common sugars, dietary fibers of plant origins, that cannot be completely broken down by the human digestive system as well as malto-oligosaccharides, which are derived from glucose monomers.

Due to high production costs rare sugars cannot be extracted from natural sources and thus have to be produced by (bio) chemical reactions. Produced by enzymatic isomerization, they are considered as added sugars, usually providing a less intense sweet taste than sucrose. Some of these compounds have a caloric content similar to sucrose but show a different metabolic profile (isomaltulose, trehalose). Others, such as allulose and tagatose are interesting because of their low caloric content (Lê, Robin, and Roger 2016).

In general, alternative sugars are used in combination with bulk sweeteners since they have lower sweetening power than sucrose when they are used as sole sweetening agent and do not provide enough bulk to the final product. An advantage of alternative sugars is the absence of any unwanted after taste (Chattopadhyay, Raychaudhuri, and Chakraborty 2014).

Allulose, an isomer of fructose, is found naturally in dried fruits. It is characterized by a low caloric content and a sweetening power of about 70% compared to sucrose. Since it is absorbed by the small intestine and excreted in urine, allulose does not affect blood glucose or insulin levels (Hossain et al. 2015). D-allulose, also known as D-psicose, was shown to possess flavor modifying properties by altering cotton candy and salted caramel flavors in water when applied in sub threshold concentrations (Smythe, Fletcher, and L. 2017).

Tagatose, an isomer of galactose, can be naturally found in small quantities in various dairy products. Its sweetening power is slightly lower than that of sucrose and it shows a high solubility. Similar to allulose, it can lower the glycemic response in humans and supports caries prevention. Both of these rare low calorie sugars are classified as GRAS by the FAO/WHO for usage in food and beverages (Mendoza, Olano, and Villamiel 2005).

Isomaltulose, an isomer of sucrose, is naturally found in honey and sugar cane. It has a mild sweet taste when compared to sucrose, and a qualitative sweetness profile comparable to sucrose. It is slowly digested which results in a positive effect on the blood glucose and insulin level (Sawale et al. 2017). In aqueous systems it is less soluble than sucrose but it is stable in acidic conditions, a characteristic of interest for yogurt production. The addition of isomaltulose to set yoghurts did not have a negative effect

on the yogurt-production process nor on pH development (Guggisberg, Piccinali, and Schreier 2011). Consistency and creaminess were increased by the addition of palatinose to a level comparable to yogurts with added sucrose but the palatinose containing samples were significantly less sweet (Guggisberg, Piccinali, and Schreier 2011).

Trehalose is naturally found in plants and fungi. Compared to sucrose, trehalose has a lower sweetening power but longer persistence of sweetness. Therefore, it is generally used in combination with other sweeteners. It is highly resistant to hydrolysis, and chemically inert in association with proteins, an aspect of particular interest to dairy industry, since industrial yogurt production often includes the addition of protein powder. Human consumption of trehalose in doses up to 50 g has been demonstrated to be safe (Richards et al. 2002).

All above-mentioned alternative sugars are considered interesting sweeteners for yogurt production (Sawale et al. 2017; Guerrero-Wyss, Durán Agüero, and Angarita Dávila 2018) and some products (e.g. Tate and Lyle, Cargill) are already on the market. Alternative sugars are also recognized to be non-carcinogenic and have obtained positive opinions from the scientific community (Lamothe et al. 2017).

8.2.2.3 *Dietary fibers*

In the European Union regulation 1169/2011 defines fiber as 'carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

- -edible carbohydrate polymers naturally occurring in the food as consumed,
- -edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence,
- -edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence'.

In fat and/or sugar reduced dairy products, the addition of various dietary fibers such as inulin, pectin, or guar gums facilitates a significant reduction in calories by compensating for the negative textural effects caused by a sugar reduction.

The evaluation of different types of carrageenan in model dairy desserts and showed that samples with l-carrageenan were perceived as sweeter and smoother compared to samples containing k and i-carrageenan (Lethuaut et al. 2005).

Esmailnejad Moghadam et al. (2019) summarized the effect of inulin on sensory properties of yogurt. The addition of inulin enhanced fruit flavor, had a positive effect on the sweetness profile and masked any undesirable taste notes. Particularly, longer chain inulin was more suitable as milk fat replacer in contrast to shorter chain inulin, which was more suitable as sugar replacer.

Isik et al. (2011) found that low-calorie yogurts containing inulin extracted from chicory root as well as polyols had acceptable rheological and sensory characteristics. In addition, the average caloric content of the tested yogurts could be decreased by about 43% compared to the original sample, without affecting the growth of the lactic acid bacteria (Isik, Boyacioglu et al. 2011).

Yogurt drinks with oligofructose added as replacement of 20% of sugar had a comparable taste and appearance to the original product (Lightowler et al. 2018). The use of oligofructose in combination with stevia, resulted in the closest sensory profile to plain yogurt containing 8% sucrose (Guggisberg, Piccinali, and Schreier 2011).

The addition of low levels (3% w/w) of Litesse, a polydextrose, improved the creaminess, mouthfeel, taste and flavor of yogurt white mass (O'Donnell and Kearsley 2012).

According to Stephen et al. (2017) dietary fibers have also blood glucose lowering and blood lipid-modifying effects. Their beneficial impact on health depends on their degree of polymerization, chemical composition and linkages. However, some negative effects of dietary fiber on sensory profile such as a sandy mouthfeel were noticed, especially at high dosages. Moreover, the consumption of high amounts of dietary fiber, particularly inulin, can sometimes lead to gastrointestinal problems, such as bloating, flatulence, and diarrhea (Boeckner, Schnepf, and Tunland 2001; Esmailnejad Moghadam et al. 2019)

These studies suggest that the addition of selected dietary fibers to compensate sugar reduction in yogurt is possible, particularly to improve the texture in low-fat yogurt.

8.2.2.4 Maltodextrins (MDs)

Maltodextrins (MDs) have a bland neutral flavor and show only a low sweetness. They are obtained from the hydrolysis of starch by heat, acids, and/or enzymes, followed by purification and spray drying steps. Various raw materials such as wheat, corn, potato, oats, and rice are potential sources of maltodextrins. Some maltodextrins are fully digestible; whereas others comprise molecules resistant to digestion, and therefore are considered as dietary fibers. For the latter, the rate of digestion and absorption is lower than for glucose due to the enzymatic pre-digestion necessary for their adsorption (Lê, Robin,

and Roger 2016). Depending on the content of dextrose equivalents, they affect different physical and chemical properties such as viscosity, stickiness, or hydrophobicity. Because of these characteristics, they are often used in sugar reduced or fat reduced products to improve textural aspects (Lê, Robin, and Roger 2016). However, maltodextrins contain the same amount of calories as sucrose (McCain, Kaliappan, and Drake 2018).

Not many studies evaluated the use of maltodextrins in yogurts, one reason being their potential 'off' flavor. Heydari et al. (2011) found that yogurts containing 3.0% maltodextrin had a dusty and drug-like 'off' flavor which was unacceptable for the majority of the panelists (n=9). Low fat vanilla yogurts produced with a mix of maltodextrin and steviol glycosides were neither appreciated by consumers (Narayanan et al. 2014).

8.3 Technological adaptations

8.3.1 Bacterial Cultures

During fermentation and storage of yogurt, bacteria added to and naturally occurring in milk perform three major biochemical processes:

- 1) Conversion of carbohydrates into lactic acid and other metabolites (glycolysis)
- 2) Breakdown of milk proteins into peptides and free amino acids (proteolysis)
- 3) Breakdown of milk fat into free fatty acids (lipolysis).

Lactic acid not only adds to yoghurt a desired acidic flavor, but also decreases the pH of milk, leading to a collapse of the casein micelles and as a consequence a coagulation of the milk. At the end of fermentation yoghurt contains 30 to 40 mg lactose and galactose per ml.

Knowledge about bacteria, in particular their metabolic activities, is important to produce fermented high quality foods, not only from a sensorial but also from a nutritional point of view. In fact, new tools such as high throughput sequencing have been developed to identify individual specific genes coding for metabolic pathways responsible for desired bacterial performances (Hansen 2002).

8.3.1.1 Starter cultures

The main purpose of the addition of lactic acid bacteria cultures in yogurt production is the formation of lactic acid, responsible for the characteristic sour taste of yogurt. The concentration of acids in yogurt is an important parameter influencing sweetness perception due to the well know interaction between sweetness and sourness perception.

Starter cultures used in yogurt production are composed of metabolically active strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. The oxygen-tolerant *S. thermophilus* starts growing first. The production of formate and pyruvate during lactose metabolism stimulates, the growth of *Lb. bulgaricus* resulting in an increase in amino acids and peptides due to its high proteolytic activity. Yogurts produced with a so-called “mild” starter culture are characterized by a lower acidity and little post-acidification during storage (Möller et al. 2007).

Nowadays, for the majority of consumers a strong acidic flavor induced by low pH values is correlated with an unacceptable taste (Harper et al. 1991; Möller et al. 2007).

8.3.1.2 Cultures with specific flavor and texturing compounds

The addition of selected bacteria strains known to produce relatively high amounts of aroma compounds such as diacetyl, acetoin and lactones which may enhance perceived sweetness intensity (White, Kilara, and Hui 2008; Chen et al. 2017).

Bifidobacteria have been widely incorporated into dairy products in the last few decades due to their potential health benefits. Bifidobacteria contribute to acetaldehyde and acetoin formation, which give the sweet-buttery notes in yogurt (Zareba, Ziarno, and Obiedzinski 2012).

Strains belonging to the genera *Leuconostoc* and *Lactococcus* are often incorporated as adjunct cultures. These strains are able to metabolize citrate resulting in the formation of diacetyl, acetic acid, and ethanol as well as diacetyl and acetoin by *Leuconostoc* and *Lactococcus* respectively (Boumerdassi et al. 1997).

In many countries, the probiotic species *Lactobacillus rhamnosus* is also added to yogurt. Although it does not significantly influence the major aroma-active volatile metabolites, it might enhance the sweetness perception indirectly by contributing to the yield of volatile organic acids and alcohols during fermentation and to an increased formation of non-volatile organic acids and free amino acids during refrigerated storage (Innocente et al. 2016). Moreover, this species is capable of producing long-chain exopolysaccharides rich in galactose which show antimicrobial properties, such as the rhamnose (Segers and Lebeer 2014). They may have a possible impact on the sweetness perception due to their chemical characteristics; however, this last hypothesis must still be proved.

Propionic acid bacteria (PAB) are used in the production of several fermented dairy products and have a potential as probiotics (Thiel et al. 2004). Mainly, PAB play an important role in the development of characteristic flavors in Swiss-type cheese due to their production of propionate, acetate, and CO₂ (Ekinici and Gurel 2008b). Besides, metabolites of probiotic bacteria, such as acetaldehyde, propionate and diacetyl, may contribute to an increase in the sweetness perception of yogurt or at least its consumer acceptance. Moreover, these bacteria grow well in set yogurt and do not compete with the development of the classic yogurt starter cultures (Ekinici and Gurel 2008a).

Metabolic engineering of lactic acid bacteria can be used to stimulate strains to over-produce certain flavor metabolites, such as acetaldehyde, diacetyl, and esters. However, it is not always possible to trace back the metabolic precursors of many of the known flavor compounds, as there are many different possibilities of flavor-forming pathways. In addition, the interactions among individual bacteria during

the process of yogurt fermentation are thought to be crucial for obtaining the desired physico-chemical product characteristics and flavors (Chen et al. 2017).

8.3.1.3 Culture with different sugar metabolic pathway

The strategy of metabolic rerouting seems to be another possible strategy that could be used for sugar reduction, since it does not require the use of recombinant DNA technology (Johansen et al. 2015).

Traditional bacterial genetic techniques have been used to redirect the metabolic pathways in *S. thermophilus* and *L. bulgaricus* in a way that the bacteria use galactose instead of the usually preferred glucose for energy production. In this specific case, metabolic rerouting was done by selecting spontaneous mutants. To induce natural mutation bacteria were grown in a culture broth containing galactose as the only sugar, resulting in a selection of strains possessing the ability to use galactose as primary energy source. The use of galactose instead of glucose for energy production increases the sweetness intensity of a yogurt, since glucose has a higher sweetening power than galactose (Johansen et al. 2015).

Interestingly, these mutant strains also show higher efficiency in fermenting lactose when compared with their parent strains, allowing for a reduction of the lactose concentration in yogurt, which in turn supports acceptability of the product by lactose intolerant people (Johansen et al. 2015).

Yogurts produced with spontaneous mutants of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* which resulted in samples with no detectable lactose, moderate levels of galactose, and high levels of glucose, were perceived as sweeter compared to samples made with a classic starter culture (Sørensen et al. 2016).

It is known that some lactic acid bacteria have the ability to divert a minute fraction of fermentable sugar such as lactose for the biosynthesis and secretion of so-called “exopolysaccharides”. These compounds consist of branched, repeated units of sugars or sugar derivatives, such as glucose, galactose, mannose, N-acetylglucosamine, N-acetyl galactosamine and rhamnose, in different ratios. Exopolysaccharides have huge application potential in the field of prebiotics, nutraceuticals, sweeteners, humectants and anti-carcinogenic drugs (Doleyres, Schaub, and Lacroix 2005). Their properties depend on their composition, type of linkage between units, length of their branches and molecular weight (Iliev, Ivanova, and Ignatova 2006). Exopolysaccharides can be classified into two groups based on their monosaccharide composition and their biosynthesis: homopolysaccharides (dextran, mutan, alternan, reuteran, pullulan, levan, inulin, curdlan etc.) and heteropolysaccharides (gellan, xanthan, kefiran) (Jolly et al. 2002).

The usage of inulin, known mostly as dietary fiber, was already discussed above (Bulking replacer: Dietary fibers). Although the interest in exopolysaccharides is very high, the sweetening power of the majority of these compounds has not been extensively studied yet. However, much more is known about their ability to act as bulking and texturizing agents, which might influence indirectly the sweetness perception of yogurt through cross-modal interactions texture-sweetness.

8.3.2 Enzymes

The usage of enzymes, added during yogurt production or encoded by specific bacteria is another possibility to influence sweetness perception of yogurt.

A well-established and often used method is lactose hydrolysis using the enzyme β -galactosidase to split lactose into its two monomers glucose and galactose (Figure 1).

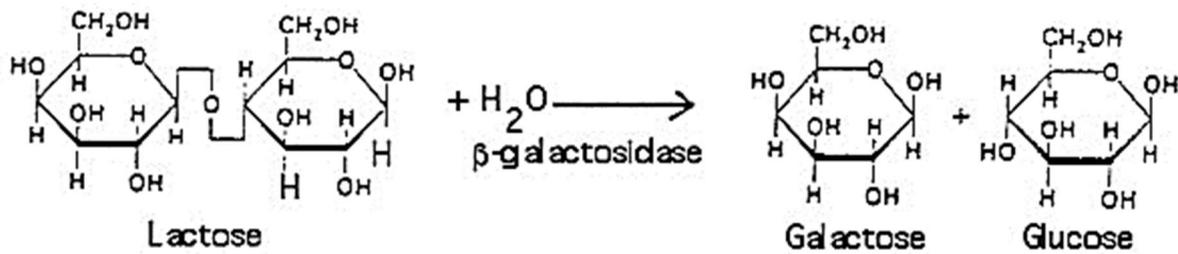


Figure 1: Enzymatic conversion of lactose operated by β -galactosidase in milk (https://openwetware.org/wiki/BISC220/S11:_Mod_1_Lab_1).

This technique has recently become more feasible thanks to improvements in the technological production of β -galactosidase (Abbasi and Saeedabadian 2015). Lactose hydrolysis is commonly obtained by adding β -galactosidase after milk acidification at 35 to 45°C, and for different periods of time depending if a partial or complete conversion of lactose is wanted (Zadow 1986). It has been proven quite a long time ago that hydrolysis of lactose in yogurt results in a milder, sweeter taste without adding other sugars (Engel 1973), although this approach leads only to a modest sugar reduction in yogurt. Indeed, according to McCain, Kaliappan, and Drake (2018), the hydrolysis of 70% of the lactose in milk increases the sweetness of milk or yogurt to the same degree as adding 2% sugar. Another advantage is its suitability for lactose-intolerant people (Tamime and Deeth 1980). In a recent study it was shown that hydrolysis of lactose also improved the sweetness and brightness of frozen yogurt (Skryplonek et al. 2017).

Lactose-reduced yogurts obtained by lactose hydrolysis, showed lower acidity (titrable acidity) than the not reduced reference products (Vénica et al. 2016). Furthermore, the addition of β -galactosidase did not affect the proportion of ketones, acids and aldehydes in the final product. In contrast, the volatile fraction found in the two product types differed depending on storage time. The observed increase in acid compounds was more pronounced in lactose-hydrolyzed than traditional yogurts (Vénica et al. 2016).

However, in the United States there is an ongoing debate about the declaration of the lactase enzyme. It is still unclear if the enzyme has to be declared as an ingredient on the label of a food. (McCain, Kaliappan, and Drake 2018).

Another enzyme of interest is the threonine aldolase responsible for the interconversion of threonine into glycine and acetaldehyde (Özer and Atasoy 2002). Acetaldehyde is the major aroma compound of natural yogurt, normally occurring in concentrations between 17 and 41 mg/L (Rysstad and Abrahamsen 1987). Acetaldehyde has been shown to have a positive impact on consumer's acceptance of yogurts: a low concentration of acetaldehyde is generally associated with a "low" flavor intensity (Ott et al. 2000). Acetaldehyde may also influence the sweetness perception of yogurts, especially when a "mild" starter culture is used, where the production of acetaldehyde is decreased in favor of the production of 2,3-butanedione and 2,3-pentanedione (Ott, Fay, and Chaintreau 1997). Glycine, formed together with acetaldehyde, might also contribute to the natural sweetness of yogurt, since a low sweetness potential is attributed to this amino acid (Kawai et al. 2012; Toko 1998). Glycine can be metabolized by bacteria since it represents an important source of energy (Konings 2002). However, no information about a possible glycine content in yogurt after addition of the precursor threonine in milk could be found (Law 1981). Methionine, another amino acid, seems to be also related to an increase in the acetaldehyde production when added to milk (Özer and Atasoy 2002), however, the metabolic pathway of uptake and production is still not fully understood.

Two proteolytic enzymes, neutrase and trypsin, were found to be most effective in modifying milk proteins through hydrolysis without causing any bitterness in the products. Hemantha Kumar et al. (2001) used neutrase and trypsin to modify milk proteins for yogurt production. Results showed improvements in sensory properties.

Enzymatic modifications can be applied not only to milk or yogurt, but also to ingredients added to them, in order to improve specific selected quality aspects of yogurt. In particular, the ability of certain enzymes to cross-link milk protein has been exploited (Ercili-Cura, Huppertz, and Kelly 2015). Routray and Mishra (2011) observed enhanced yogurt firmness, water-holding capacity, growth rate of bacteria, and yogurt flavor by enzymatic modification of milk proteins.

Therefore, the use of enzymes other than lactase in sugar-reduced yogurts may optimize the texture but not much is known about their effects on flavor.

8.3.3 Modifications of matrix structure

A further possible strategy for a sugar reduction, without changing the level of perceived sweetness intensity, would be to influence the matrix structure and, in consequence, facilitate the release of sweet flavor molecules. The production of stirred yogurts where the matrix is partially broken down represents an application of this type of strategy.

8.3.3.1 *Spatial distribution/ layering of sucrose*

A recent strategy to improve the sweetness perception is an induced non-homogeneous distribution of the sugar particles within the product. Holm, Wendin, and Hermansson (2009) have studied the effect of the sugar distribution in gelatine layers on sweetness perception. Gelatines differ from food thickeners in their melting characteristics in the mouth. The presence of sugars increases the strength of gel network of gelatine.

An inverse relationship between gel hardness and overall gel flavor has been reported. The addition of pectin to a gelatine gummy gel increases the fruity, sweet, and tart flavor of the gels. The increased flavor perception at higher pectin concentrations could potentially be accounted for by the difference in texture (more firm and hard) or by the inherent fruity flavor of pectin (DeMars and Ziegler 2001). Mosca et al. (2012) tested sucrose gels in several layers varying in textural properties, obtained by adding agar and gelatine. The product containing sucrose in layers was perceived sweeter than the one containing homogeneously distributed sucrose. These authors suggested that the breakdown behavior of the gel matrix during oral processing affects the perception of sweetness of layered gels.

8.3.3.2 *Microencapsulation*

Microencapsulation is a technique that consists in trapping a compound into a capsule composed of an organic polymer. Specifically, the aim of microencapsulating sweeteners is to increase the sweetener's fluidity and resistance to high temperatures and prolong the sensation of sweetness by controlling sweetener release in the mouth (Gouin 2004). In yogurt production, the stability of sweeteners is not a critical factor as it requires temperatures of 43°C but microencapsulation of sugar may promote the sweetness perception of the yogurt. The membrane of a microcapsule in yogurt has to be composed of a material that has good emulsifying properties, film-forming, low viscosity, stability, absence of flavor, good protection of the core content at low pH and affinity to the yogurt matrix (hydrophobicity). Microcapsules are usually produced by coacervation of gelatine, spinning disk, extrusion, spray drying, or

liposome entrapment (Gouin 2004). The capsules are usually insoluble in water, resistant to high temperatures and show excellent characteristics for controlled release (Dong et al. 2011). Of the two studies found on microencapsulation of sweeteners neither was done with yogurt. One study focused on the stability of encapsulated aspartame in cakes (Wetzel and Bell 1998), while the other evaluated the stability of microencapsulated aspartame in water (Rocha-Selmi et al. 2013).

Although only few data are available for sugars, microencapsulation could be considered as an innovative strategy to increase sweetness perception in yogurt. However, the stability of a microcapsule in an acidic semi-liquid environment like yogurt is a challenging task and has to be examined further as the material used to encapsulate sweeteners in yogurt should preferably be of dairy origin.

8.3.3.3 Size of the fruit pieces

In the case of fruit-based yogurt, usually produced as stirred yogurt, the size of the fruit pieces might play an important role on the sweetness perception and consumer acceptance of such a product. The ratio between white mass and fruit pieces is an important parameter in the selection of fruit yogurts. Big parts of fruits can retain sugars from the fruit mass processing and the fructose contained in the fruit, creating a layering effect when in contact with the taste receptor, masking the sour flavor deriving from the white mass. The addition of fruit preparations in yogurts could also help to modify how long a product stays in the mouth, intra-oral manipulation of the product and aroma release.

Mesurole et al. (2013) studied the impact of size and firmness of fruit pieces in fat-free pear yogurts. The yogurt with the hardest pear pieces was perceived as having a more intense "green" note and less intense "caramel" note, but no significant taste difference was observed. Yogurt with the largest pear pieces tended to be perceived as less sweet and "fattier" than the standard one. The size of pear pieces did not have a significant impact on aroma perception.

Creating differences in spatial sugar distribution in a yogurt may be an innovative way of reducing sugar but until now, only the use of different size and firmness of fruit pieces was documented for yogurt.

9 General Conclusions

There is a broad range of approaches to reduce the amount of added sugar yogurt. The sequential “silent” reduction of sugar is particularly interesting, since usually there is no need to reformulate the product. When implementing a sequential sugar reduction strategy, the concentration steps by which sugar can be reduced have to be experimentally determined for each product so that the consumer does not notice the difference in sweetness intensity. Moreover, no information is available on the time necessary to wait until a next reduction step can be done. Most likely, successive reduction steps only be performed to a certain concentration limit, since the reduction of sugar results also in a decrease of perceived aroma intensity and might also change yoghurt texture. For example, aromas such as vanilla or strawberry, which are frequently used to aromatize yogurts, are perceived stronger in the presence of sugar. Sugars also influence the unfolding and aggregation of proteins, gelatinization as well as the creaminess of the yogurt. Therefore, especially in low sugar concentration ranges a consistent reduction of sucrose in yogurt without any type of sweetness compensation reformulations are necessary in order to avoid a decline of consumer acceptance.

Sugar replacers, especially high intensity sweeteners are often used in dairy industry to compensate a sugar reduction in yogurt, because the sweetness intensity level of the original product can be maintained. The sweetening power of sugar replacers is usually much higher than sucrose. However, they often vary in their sweetness quality (e.g. undesirable off flavors) as well as temporal perception.

Sugar replacers also vary in their stability to heat and to a low pH of the product, which is in the case of yogurt a crucial factor to consider. Furthermore, sugar reduced products containing sugar replacers often show a lower calorie content. However, the application of sugar replacers has been strongly debated in terms of potential long-term health impacts. Considering the ongoing trend towards natural products the application of sugar replacers in yoghurts might also influence consumer acceptance.

Sugar alcohols, dietary fibers and maltodextrins usually are used as bulking agents since they have a lower sweetening power than sucrose. Sugar alcohols represent an interesting group of substances for the replacement of sucrose because of their low glycemic response. However, only a few studies on sugar alcohols in yogurt have been carried out. The use of maltodextrins is challenging because of their tendency to produce ‘off’ flavors. Furthermore, also the application of dietary fibers such as inulin has

to be carefully evaluated since their use cause is often related to undesirable side effects like gastrointestinal problems.

Odor-induced taste enhancement is a strategy based on specific aroma–taste associations that can amplify the sweetness perception also at a cognitive level. In theory, this strategy allows sugar reduction without any theoretical loss of sweetness intensity or increase of energy intake. In order to successfully apply this strategy in food products a combination of compounds and concentrations has to be found that maximizes taste (sweetness) and minimize undesirable sensory effects. Since aroma-induced taste enhancing depends on aroma and product, specific screening tests are necessary for each product. According to the literature, fruity aromas such as strawberry, peach, apple or caramel and sweetness enhanced sweetness of dairy products.. Results found in literature about the effect of vanilla aroma were incongruent.

Although, the usage of aromas seems to be an interesting strategy to compensate a reduction in sweetness intensity only a moderate effect on reduction of sugar was achieved. However, when combined with other cross-modal interactions, aromas may be able to produce bigger effects compensating for a decrease in sweetness perception.

Sweetness perception can also be influenced by the addition of proteins. The latter results in changes of the food matrix structure, which in turn influences the flavor perception and textural attributes such as creaminess of a yogurt:

However, excessively high protein concentrations in yogurt can provide an off-flavor and a sandy mouth-feel. Also the addition of sweet peptides like monellin could be an interesting way to compensate a sweetness loss due to sugar reduction. However, only few studies have been conducted with sweet peptides in yogurt. In addition, these substances are still expensive and only a few sweet peptides are recognized as safe.

Another possible strategy is the treatment with enzymes of the milk used for yogurt production. Enzymes can be used to convert lactose that has a low sweetening power into its monosaccharides, which have a higher sweetening power, or for the production *ex-novo* of sweetening compounds not usually found in yogurt. The hydrolysis of lactose, however, gives rise to only a small increase of sweetness.

Research on bacterial cultures used in yogurt production is an alternative approach to reduce added sugar content. A technique already implemented in industrial yogurt production is the use of a mild starter culture to decrease the acidity level of yogurt. Due to the well-known interaction between perceived sourness and sweetness, the sweetness intensity of the yogurt increases.

Addition of propionic acid bacteria to the classic yoghurt bacteria *S. thermophilus* and *L. bulgaricus*, may enhance the production of flavor compounds through synergistic metabolic activities of the strains. However, knowledge on synergistic interactions among bacterial strains is scarce and more research is needed to understand metabolic activities on specific bacteria communities.

By selection of bacterial strains showing desired fermentation characteristics, using genetic engineering, it is possible to produce natural sweetening compounds acting as sugar replacers. However, DNA recombinant techniques lead to genetically modified microorganisms (GMO) which have a strong negative image in public. Spontaneous mutants obtained by random mutagenesis is another possibility to “design” bacteria strains with desired metabolic properties. This genetic technique does not directly recombine the genome of the microorganism, but rather pushes the adaptation of the bacteria strains to new unfamiliar environmental conditions. This technology was already used to create a yoghurt starter culture, which uses galactose instead of glucose as an energy source resulting in a sweeter product. In the future for example strains could be promoted that are able to produce sweetness enhancing flavor compounds or sugars having a higher sweetening power than sucrose. However, for the selection and implementation of adequate bacteria strains much more research is needed and long time periods have to be foreseen.

Finally, sweet taste could also be enhanced in dairy products through texture modifications and/or non-homogeneous distribution of taste stimuli in the matrix like layering or microencapsulation. However, only a few studies about the use of such techniques have been applied to yogurt. Thus, estimation of the effect on these technological methods on sugar reduction is difficult.

10 Literature

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