

## Maltose is an inappropriate indicator of digestibility of complementary foods containing substantial amounts of this simple sugar

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### Abstract

The *in vitro* starch digestibility (IVSD) method (“as-is” or modification) was used to assess the digestibility of two sweetpotato-based complementary food (CF), denoted orange-fleshed ComFa and cream-fleshed ComFa, and two cereal-based CF: Cerelac (wheat-based commercial infant cereal) and Weanimix (maize-soybean-groundnut blend). Using the IVSD method (“as-is”), the sweetpotato formulations with high maltose (averaging 22.24 g/100 g) and low starch, about 15.15 g/100 g, had far lower digestibility values of 6.29 g/100 g, a quarter of that for Weanimix, which contained maltose and starch at levels of 2.72 g/100 g and 48.38 g/100 g, respectively. Further, the IVSD method employed “as-is” estimated the digestibility of Cerelac to be 11.53 g/100 g, about half the value for Weanimix. Conversely, for the modified method, the sweetpotato-based formulations had estimated digestibility value about 3 times higher than Weanimix (63.91 g/100 g), and 1.5 times higher than Cerelac (117.76 g/100 g). The IVSD method (“as-is”) gives false negative results when used to estimate the digestibility of CF that contain significant amount of endogenous maltose. Therefore, its application to predict the suitability of CF warrants further validation.

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### Introduction

The ease of digestibility of food invariably leads to the bioavailability of energy and nutrients required to sustain life. Carbohydrates such as mono- and disaccharides (simple sugars), as well as starch and its hydrolysed products could constitute about 60-75% of the total macronutrients of plant-based complementary foods (CF) (Codex Alimentarius Commission, 1991). The *in vitro* starch digestibility (IVSD) is therefore used by researchers to estimate the digestibility of foods (Gahlawat and Sehgal, 1994; Kshirsagar *et al.*, 1994; Gahlawat and Sehgal, 1998; Altan *et al.*, 2009) as carbohydrates constitute a significant portion of macronutrients in plant-based CF. An IVSD method usually cited to estimate the digestibility of foods is that published in 1982 by Singh *et al.* (1982).

The IVSD method was slightly modified and patented in 2002 (Hansson and Spégel, 2002); the inventors used the method to evaluate the digestibility of different native starches but not a whole food matrix, suggesting that the method may

not be appropriate for estimating the digestibility of foods. However, the IVSD method has been used to estimate the digestibility of infant foods either directly (Gahlawat and Sehgal, 1994; Kshirsagar *et al.*, 1994; Gahlawat and Sehgal, 1998) or with some modification (Onyango *et al.*, 2004; Nandutu and Howell, 2009). The modification, which expresses the IVSD of the CF as a ratio of the maltose released to the starch content rather than the whole food matrix, is practically recommended because of the specificity of  $\alpha$ -amylase for complex carbohydrates (Lebenthal *et al.*, 1982).

It is recommended that foods for infants should have starch in small quantities as its hydrolysis improves with age (Weaver, 2000; Lentze, 2008); the presence of a significant proportion of undigested and unfermented dietary starch in the faeces of 5-37 month-old infants and young children (Verity and Edwards, 1994) drives the recommendation for the quantity of starch in CF. The ability for infants to ferment complex carbohydrate is not fully developed until about 8 months old (Parrett *et al.*, 1997). It is has been reported that for infants, fermentation

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of undigested starch by colonic flora is less, thus scavenging and absorption of short-chain fatty acids (predominantly butyrate) and their utilisation as a source of energy (Tester *et al.*, 2004) is compromised. Therefore, CF that contain significant amount of carbohydrate as starch may actually have less bioavailable energy than as estimated based on compositional analysis.

The inability of infants to digest starch in large quantities may be due to the low levels of both salivary and pancreatic amylase (Lebenthal *et al.*, 1982; Christian *et al.*, 1999), that are required to initiate the sequential breakdown of starch into oligomers, maltose and glucose (Dona *et al.*, 2011). Salivary amylase only reaches adult levels from 6-12 months (Rossiter *et al.*, 1974), while pancreatic amylase has full activity only from 5-12 yr (Gillard *et al.*, 1983). In contrast, infants are born with full activity of the brush-border enzymes (Lebenthal and Tucker, 1986; Lentze, 2008), which suggests that they are physiologically ready to hydrolyse sugars and degraded starch oligomers better than starch (Weaver, 2000). Needless to say, a complementary formulation with significant of carbohydrate as simple sugars should have higher digestibility (Malunga *et al.*, 2014).

To improve the digestibility of CF, an enzymatic pre-digestion of cereals is recommended in all the Codex Standard Guidelines published or in-preparation (Codex Alimentarius Commission, 1991; Food and Agriculture Organization and World Health Organization, 2011, 2012; Malunga *et al.*, 2014). An industrial CF manufacturer, Nestlé®, refer to the cereals used in their products as “Cereal Hydrolysed Enzymatically (CHE)”, and it is stated on the package that CHE products are easy to digest by infants (Nestlé, 2006). CHE infant cereals have starch molecules that have been hydrolysed into dextrans and reducing sugars (example, maltose and glucose) (Codex Alimentarius Commission, 1991; Food and Agriculture Organization and World Health Organization, 2011, 2012). Hence, a quantification of maltose could indicate the digestibility of CF.

There may be a flaw in using the IVSD to predict the digestibility of pre-digested foods as recommended in the Codex Guidelines (Codex Alimentarius Commission, 1991; Food and Agriculture Organization and World Health Organization, 2011, 2012; Malunga *et al.*, 2014) for CF, as the method includes the “actual sample” in the blank assay. The reason for the suggested flaw is that the blank could have substantial maltose, if the products contained measurable level of this disaccharide, leading to a false-negative IVSD value.

In this study, the IVSD method was used to estimate the digestibility of CF with varying levels of endogenous maltose and starch, processed either from sweetpotato, maize or wheat and other ingredients.

## Materials and Methods

### *Complementary foods*

Four ready-to-eat CF: cream-fleshed and orange-fleshed ComFa (sweetpotato-based products); Weanimix (a maize-soyabean-groundnut blended food); and Nestlé® Cerelac® infant cereal wheat and ikan bilis (Nestlé, Malaysia) made up as previously published (Amagloh and Coad, 2014) were assayed using the IVSD method as done by other researchers on different types of CF (Gahlawat and Sehgal, 1994; Kshirsagar *et al.*, 1994; Gahlawat and Sehgal, 1998; Onyango *et al.*, 2004; Nandutu and Howell, 2009) to estimate their digestibility.

### *Chemical analysis*

The method published by Singh *et al.* (1982) was followed and was also modified to estimate the digestibility of the infant formulations mentioned above. The modification entailed the choice of Milli-Q water instead of sample as reported by Singh *et al.* (1982) for the blank assay to correct the absorbance readings of the in vitro digested foods.

Approximately 50 mg of the defatted samples were weighed directly into 2-mL polypropylene tubes and 1.00 mL phosphate buffer (0.20 M; pH=6.9) was added and capped, and allowed to stand overnight. The sample suspensions in the phosphate buffer were gently mixed and 0.50 mL porcine pancreatic  $\alpha$ -amylase (A6255, Sigma-Aldrich; activity=1333 units/mg protein) solution prepared by dissolving 25 mg of the  $\alpha$ -amylase suspension in 62.50 mL of the phosphate buffer. The sample suspensions containing the  $\alpha$ -amylase were incubated in a water bath (Heto Birkerød, Denmark) at 37°C and gently shaken for 2 h. This was followed by the immediate addition of 2.00 mL of the coloured reagent and the tubes were placed in boiling water for 5 min. The coloured reagent was prepared from 3, 5-dinitrosalicylic acid (D0550, Sigma-Aldrich) and sodium potassium tartrate tetrahydrate [BSPPS303, Biolab (Aust) Ltd] in 2 M sodium hydroxide.

After 5 min in the boiling water, the tubes were removed, the caps slightly unscrewed, and placed in a refrigerator (4°C) for 30 min to cool. The cooled sample suspensions were filtered into a 100-mL volumetric flask and made up to volume using Milli-Q water. Absorbance was measured at 540 nm

using UV/Visible spectrophotometer (Pharmacia LKB Ultrospec II, England). Sample blanks were prepared in a similar way except the addition of  $\alpha$ -amylase was done after the coloured reagent had been added to the suspensions after incubation for 2 h. The absorbance measurements of both the actual sample and reagent blank (actual sample included in the preparation) were corrected for the absorbance of the coloured reagent. An equivalent amount of Milli-Q water as sample was weighed and run as reagent blank. Sample and blank were both run in parallel, and each sample with its respective blanks was assayed five times.

About 10 mg, in six replicates, of maltose monohydrate (M5885, Sigma-Aldrich) were weighed and the IVSD as described above (“as-is” and modification) were used to determine the percentage recovery of maltose. The absorbance of the test samples and the maltose recovery assays were used to determine the maltose levels from a calibration curve prepared from maltose monohydrate (M5885, Sigma-Aldrich) with seven concentration levels (10, 20, 40, 60, 80, 100 and 120 mg/L) in triplicates. Two millilitres of the coloured reagent were added to each of the concentration levels and placed in boiling water for 5 min. The standard solutions were cooled in a refrigerator for 30 min, and then varying amounts of Milli-Q water were added to obtain a final volume of 25 mL. The coefficient of determination ( $R^2$ ) of the calibration curve was 0.99, indicating very strong linearity.

#### Statistical analysis

The means of the IVSD of the CF were compared by one-way analysis of variance (ANOVA) with the use of Minitab® 16.2.2 (Minitab Inc., State College, PA, USA) for each parameter assayed. Tukey’s studentized range test was used to compare differences between means when the ANOVA result was significant ( $P < .05$ ). The data generated from the recovery of maltose were compared using two-sample t-test. All results are expressed as means with their standard deviations.

## Results and Discussion

The levels of maltose and starch in all the CFs are presented in Table 1, and have been previously discussed (Amagloh and Coad, 2014). The high maltose content in the ComFa formulations is due to their higher endogenous  $\beta$ -amylase content than cereals (Mao and Sakai, 2005). When sweetpotato is heated at 65°C or above, the  $\beta$ -amylase converts most of the starch to maltose (Walter *et al.*, 1976;

Table 1. Maltose and starch contents (g/100 g dry matter basis) of sweetpotato- and cereal-based CF

Complementary food	Maltose (g/100g)	Starch (g/100g)
<b>Sweetpotato-based</b>		
Cream-fleshed ComFa	24.10±0.54 <sup>a</sup>	17.11±0.22 <sup>c</sup>
Orange-fleshed ComFa	20.38±0.41 <sup>b</sup>	13.18±0.20 <sup>d</sup>
<b>Cereal-based</b>		
Cerelac	10.31±0.12 <sup>c</sup>	30.93±0.34 <sup>b</sup>
Weanimix	2.72±0.12 <sup>d</sup>	48.38±0.50 <sup>a</sup>
	$P < 0.0001$	$P < 0.0001$

Values are means  $\pm$  standard deviation (n=3); Values with the same superscript letter (a, b, c, d) in a column are not significantly different ( $p > 0.05$ ); Adapted from Amagloh and Coad (2014).

Mao and Sakai, 2005; Ridley *et al.*, 2005). Although, the sweetpotato CF was not predigested, the level of maltose was significantly higher than Weanimix and Cerelac, approximately 8- and 2-times, respectively. The ratio of starch to maltose was about 17.79 in Weanimix; 3.00 in Cerelac; 0.71 in cream-fleshed ComFa and 0.65 in orange-fleshed ComFa, predicting that the digestibility of the ComFa products would be markedly higher than the cereal-based formulations.

The prediction was deduced from the recommendation that pre-digestion of starch into dextrans and reducing sugars such as maltose improves the digestibility of CF (Codex Alimentarius Commission, 1991); and also the suggestion that starch should be in CF in small quantities as its hydrolysis improves with age (Weaver, 2000; Lentze, 2008). The prediction and suggestion may be due to the low levels of both salivary and pancreatic amylase (Lebenthal *et al.*, 1982; Christian *et al.*, 1999), which are required to hydrolyse starch into oligomers, maltose and glucose (Dona *et al.*, 2011) as stated earlier.

Applying the method published by Singh *et al.* (1982) “as-is”, the IVSD for Weanimix was significantly higher by percentage differences equalling 124, 127, and 82 compared with cream-fleshed ComFa, orange-fleshed ComFa and Cerelac respectively (Table 2). This is physiologically difficult to explain because maltose, which is one of the intermediate metabolites during starch hydrolysis (Codex Alimentarius Commission, 1991), was significantly higher in the ComFa products and Cerelac compared with Weanimix (Table 1). As maltose is a product of the hydrolysis of starch by amylase, the lower IVSD for the ComFa and Cerelac may be due to product inhibition by the disaccharide on the activity of the enzyme. This finding indicates that using the IVSD “as-is” method may not be appropriate if the food matrix contains substantial amount of endogenous maltose as would be present in heat-processed sweetpotato-based products (Ridley

Table 2. *In vitro* starch digestibility values of sweetpotato- and cereal-based CF assessed with and without modifications of referenced method and the ratio of the “sample” to “blank” assay values

Complementary food	“As-is” method*	Modified method†		Ratio of maltose quantified from “sample” to “blank” assay‡
		Starch content of sample weighed	Whole sample weighed	
<b>Sweetpotato-based</b>				
Cream-fleshed ComFa	6.46±2.52 <sup>c</sup>	174.55±10.53 <sup>b</sup>	29.84±2.00 <sup>b</sup>	1.21±0.09 <sup>b</sup>
Orange-fleshed ComFa	6.12±1.42 <sup>c</sup>	229.69±5.34 <sup>a</sup>	30.30±0.42 <sup>b</sup>	1.19±0.05 <sup>b</sup>
<b>Cereal-based</b>				
Cerelac	11.53±3.11 <sup>b</sup>	117.76±6.71 <sup>c</sup>	36.43±2.01 <sup>a</sup>	1.35±0.11 <sup>b</sup>
Weanimix	27.40±1.83 <sup>a</sup>	63.91±3.77 <sup>d</sup>	30.98±1.62 <sup>b</sup>	3.30±0.22 <sup>a</sup>
	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001

Values are means ± standard deviation (n=5); Values with the same superscript letter (a, b, c, d) in a column are not significantly different (*p* >0.05);

\*“As-is” was the method published by Singh *et al.* (1982), which correct for value of maltose quantified from a sample with the same sample included in a blank assay but addition of enzyme to the blank is after the incubation period. Expression of the *in vitro* starch digestibility values was per amount of sample weighed.

†Modification included the used equivalent amount of Milli-Q water instead of the sample for the blank assay to correct for corresponding values of sample; and also the calculation of the *in vitro* starch digestibility value as ratio of the maltose quantified to the amount of starch contained in each sample.

‡Assay done using the “as-is” method.

*et al.*, 2005; Amagloh *et al.*, 2013; Amagloh and Coad, 2014) or as in commercially-processed infants cereals like Cerelac (Nestlé, 2006).

The major limitation using the “as-is” method is the use of sample in the blank assay to correct for the IVSD for the actual assay. Comparing the “sample-assayed” quantified maltose to the “blank-assayed” maltose (Table 2), it was observed that the samples with substantial endogenous maltose (cream-fleshed and orange-fleshed ComFa, and Cerelac) had ratios, approximately 2.5 times, significantly lower than that of Weanimix (Table 1). This finding indicates that the “blank” used in the “as-is” method is inappropriate, as the maltose quantified from the “blank” would not be very different if the sample investigated contains significant amounts of endogenous maltose.

Modification of the IVSD method using Milli-Q water instead of sample for the “blank” quantification of maltose to correct for the absorbance measurement of the “sample” expectedly resulted in the samples with substantial endogenous maltose having significantly higher IVSD values when expressed in terms of the starch they contained than using the whole sample weighed (Table 2). Because human infants are physiologically ready to hydrolyse sugars and degraded starch oligomers better than starch (Weaver, 2000), the authors predicted the samples with higher maltose to have higher IVSD.

Contrary to the IVSD obtained when the expression was in terms of whole sample weighed for the assay, expectedly Cerelac had significantly higher score, about 1.2-times, than that of Weanimix (Table 2). Although Cerelac contains relatively less amount

of maltose than the sweetpotato-based CF, the IVSD of Cerelac was unpredictably higher. As postulated earlier in the Introduction that expressing IVSD in terms of the starch content is not physiologically justifiable; it was this calculation that gave the expected results. Samples with higher endogenous maltose should have higher digestibility when maltose is the index of digestibility (Codex Alimentarius Commission, 1991; Food and Agriculture Organization and World Health Organization, 2011, 2012; Malunga *et al.*, 2014). As  $\alpha$ -amylase has specificity for complex carbohydrate (Lebenthal *et al.*, 1982), the expression in terms of starch content is warranted. However, the authors suggest that the starch should not be isolated from the food matrix and used for the assay to predict digestibility as the interference of  $\alpha$ -amylase action by dietary fibre, plant cell walls or their constituents, and antinutritional factors such as tannins and phytate (Snow and O’Dea, 1981; Alonso *et al.*, 2000; Kumar *et al.*, 2010; Yun *et al.*, 2010; Dona *et al.*, 2011), would be ignored.

Maltose recovery using 10 mg maltose monohydrate, determined on 6 replicates, indicated that the “as-is” method gives false-negative results, as no recovery of maltose was observed (-4.87±4.67%), pointing to a flaw in the method. However, using Milli-Q water as blank instead of sample, a recovery of 91.00±2.99% of the maltose assayed was recovered. Clearly, the IVSD “as-is” method underestimates digestibility if a food matrix contains significant amount of maltose.

As can be deduced from the name of the method IVSD, the method was probably developed to be

used to determine the digestibility of native starches (Hansson and Spégel, 2002), and not to apply to whole food matrices. The most appropriate way would be to determine the starch content of the sample and express the maltose released in terms of that. Notwithstanding the suggested way to predict digestibility using IVSD, the method may be inappropriate to assess the digestibility of CF due to the insignificant role of amylase in carbohydrate digestion during infancy (Lebenthal and Tucker, 1986; Christian *et al.*, 1999; Lentze, 2008;). In any case, in infants with no carbohydrate metabolism disorders, it is expected that digestibility of simple sugars should be easier than whole starch.

Nonetheless, when IVSD is to be used to estimate the digestibility of CF, the ratio should be maltose released to the starch present in the sample. The starch should not be extracted for the IVSD assay. The “blank” assay described in the method that involved using the food sample to which amylase is added after the 37°C incubation period is not appropriate when used for formulations containing significant amounts of maltose as carbohydrate. This “blank” leads to false negative results, we suggest the use of Milli-Q water instead as it is generally accepted for blank assay in analytical studies.

## Conclusion

The IVSD method does not precisely estimate the digestibility of CF when the food matrix contains a significant amount of endogenous maltose, thus this method warrants further investigation. As the IVSD method uses maltose as an index for digestibility, quantification of the level of this disaccharide could be used to assess the suitability of CF for infant feeding.

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