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Comparative study on the effect of *Thaumatococcus daniellii* (Benn) Benth sweetener on the Physicochemical and Sensory Properties of Sorghum based *Kunun-zaki* Drink

***[1]OJO, A; [2]ENUJIUGHA, VN; [2]AYO-OMOGIE, HN; [3]ABIODUN, OA**

*[1]Department of Food Science and Technology, Osun State Polytechnic, Iree, Nigeria, [2]Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria, [3]Department of Home Economics and Food Science, University of Ilorin, Ilorin, Kwara State, *ayo_ojo62@yahoo.com, Funmiabiodun2003@yahoo.com*

ABSTRACT: Effect of a natural sweetener (*Thaumatococcus danielli*) on the physicochemical properties and sensory evaluation of *kunun-zaki* was determined. Proximate and mineral compositions of the natural sweetener, physicochemical properties and sensory evaluation of *kunun-zaki* using *Thaumatococcus danielli* and sucrose were determined. Proximate composition of the aril showed that protein had (33.03%), crude fibre (5.20%), ash content (4.79%), moisture content (12.20%), fat content (0.16%), and carbohydrate (44.17%). The result of mineral obtained for the aril showed the following values potassium (190.00ppm), sodium (167.66ppm), calcium (132.96 ppm), iron (21.59 ppm) and magnesium (14.40 ppm). Physicochemical composition of *kunun-zaki* varied with concentrations of *Thaumatococcus danielli* aril and sucrose pH ranged between (3.90-4.90), total solid (4.95-13.49 %) and titatable acidity (0.78-0.39 %) for *Thaumatococcus danielli* while *kunun-zaki* sweetened with sucrose had pH (3.51-4.90), total Solids (4.95-7.43%) and titratable acidity (0.74-0.85) respectively. The sensory evaluation showed that the samples sweetened with *Thaumatococcus daniel* aril compared favorably with sucrose in terms of colour, taste, aroma and overall acceptability.
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Keywords: *Kunun-zaki*, Proximate, Mineral, Sensory properties, *Thaumatococcus danielli,*

Kunun-zaki is a traditionally fermented non–alcoholic beverage that originated from the northern part of Nigeria, which can be produced either from millet, sorghum or maize. *Kunun-zaki* is a Hausa word meaning "sweet beverage", it is now widely consumed in several parts of Nigeria owning to its refreshing qualities (Osuntogun and Aboaba, 2004). *Kunun-zaki* is generally acceptable and used as substitute for alcoholic drinks (Gaffa and Ayo, 2002). There are several types of *kunun-zaki* but few have been reported in literature; *kunun-zaki, kunun-tsamiyat*, *kunun-gyada* (Nkama *et al*., 1995) and *kunun-akamu* (Oyeyiola, 1991). Others include *kunun-baule, kunun-jiko* and *kunun-gayamba* (Amusha and Ashaye, 2009). *Kunun-zaki* is the most preferred among the *kunun* types, reported in the literature Gaffa *et al*. (2002). Although production of *kunun-zaki* is in small scale level, the beverages are widely sold in the local market and at resorts. Gaffa *et al*., (2002) reported that 73% of the population in Nigeria consumes *kunun-zaki* daily and 26% occasionally. The product cost less compared to conventional carbonated beverages but the products are not usually properly packaged. The product is offered in cups, transparent polyethylene sachets, plastics and glass containers for retail sales. Consumption is all year round with peak production in the season (February–July) when the beverage is served preferably chilled. The production process of *kunun-zaki* is done with local household utensils with processes varying between individuals, households and localities (Adeyemi and Umar, 1994)

The use of natural sweeteners in food products is encouraged in food industry. Artificial sweeteners like saccharin, aspartame, cyclamate and Acesulfame K are used world-wide as low caloric sweeteners by patients affected by disease linked to the consumption of sugar, e,g diabetics, hyperlipemia ,caries, obesity etc. but they have side effects such as psychological problems, metal disorders, bladder cancer, heart failure and brain tumors (Hagiwara *et al.,* 1984; Nabors, 1988; Kanarek, 1994; Cohen, 2001; Weihrauch *et al*., 2002). Sweet protein have the potential to replace these artificial sweeteners by acting as natural good low calories sweeteners , as we know proteins do not trigger demand for insulin in the patients whereas sucrose does. There are seven known sweet and taste modifying proteins, namely Brazzein, Thaumatin, Curculin, Mabinlin, Miraculin and Pertaclin (Faus, 2000). Thaumatin is a low calories intensely sweet- tasty protein (Raimi *et al*., 2011) and flavour modifier (Green, 1999). It consists of 207 amino acids with eight intramolecular disulfide binds and contains no free cysteine residues (Kant, 2005). The aril is of an intensely sweet, non-toxic and heat stable protein. Thaumatin is extracted, used as desserts, chewing gums and pet foods. The seeds of *Thaumatococcus danielli* also produce a jelly that swells (entrap water) to 10 times its own weight and hence provide a substitute for agar (Yeborah *et al.,* 2003). The thaumatin are first sweet–tasting proteins that have been found in nature and the crystals are about 2000-3000 times sweeter than sucrose and neither allergic nor mutagenic or teratogenic (Yeborah *et al*., 2003). Thaumatin has

**Corresponding author email: *ayo_ojo62@yahoo.com, Funmiabiodun2003@yahoo.com*

been approved for use in many countries as both a flavor enhancer and a high- intensity sweetener (Zemanek and Wasserman, 1995). Therefore, this work determines the effect of a natural sweetener (*Thaumatococcus danielli*) on the physicochemical and sensory properties of *kunun-zaki.*

MATERIALS AND METHODS

*Materials***:** Pearl millet, sorghum, sucrose were purchased from Sabo market in Osogbo, Osun State. *Thaumatocuccusdanielli*was obtained from a farm at Esa–odo,Osun State, Nigeria.

*Methods, Production of kunun-zaki***:** The modified method of Ayo *et al.* (2013) was adopted for processing of kunun-zaki. One kilogram (1 kg) of cleaned sorghum grains were washed and steeped in clean water for 48 h to soften the seed. The grains were washed to remove stones and wet milled and no spices were added into the slurry. Two-third of the slurry was mixed with 2500 ml of boiling water and stirred to form a gel; this was allowed to cool for 3 h. The remaining one-third of the slurry was added to the gel, mixed with cold boiled water (1000 ml) and left open to ferment for 12 h). It was then sieved with a muslin cloth and the filtrate was sweetened with miracle fruit (*Thaumatococcus danielli*) and sucrose. The sweetener (*Thaumatocuccus danielli*) and sucrose were added to *kunun zaki* at different concentrations (2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 g) while other ingredients were maintained at constant level.

ANALYSES

*Proximate Analysis***:** *Determination of Moisture Content***:** A clean glass Petri-dish to be used was oven dry for about 30 minutes, and then cooled in desiccators. The cooled Petri-dish was weighed as W1 and 2 g of the sample was placed into the Petri dish and heated in the oven at 105 ^{o}C for 5hours, this was done until a constant weight obtained. Then the moisture content of sample was calculated.

*Determination of Crude Protein***:** Sample (2 g) was weighed into the Kjedahl digestion flask to ensure that all sample material get to the bottom of the flask. Kjedhal catalyst (0.5 g copper sulphate and 10 g sodium sulphate) was added to the sample and 25ml of concentrate sulfuric acid was also added to sample and then fixed for 3hours in the digestion unit. The digest, a pure green solution after cooling was then change into a colourless liquid that was transferred into 250ml volumetric flask and made up to mark with distilled water.

5ml portion of the digest above was pipette into the body of the apparatus via the small funnel aperture followed by 10ml 0f 40% NaOH into the same funnel, 20ml of 4% boric acid indicator was pipette into conical flask and left to heat. The steam exit off the distillatory was closed and the change of colour of boric acid indicator to green was timed. The mixture was distilled for 15min in accordance with (AOAC, 1990). The green colour solution obtained was then titrated against 0.01N HCL contained in a 50ml burette. At the point, the green colour turns to blue which indicate that all nitrogen trapped as ammonia borate [$(NH_4)_2BO_3$] have been removed as ammonium chloride (NH_4CL). The protein content was calculated from the relationship below

$$Total\ protein\ (\%) = \frac{Titre\ value\ x\ Normality\ of\ acid\ x\ 0.014\ x\ 250\ x\ 100}{Ml\ of\ digest\ x\ Wt\ of\ sample}$$

*Dermination of Ash Content***:** Sample (2 g) was weighed into a porcelain crucible with known weight and the weight was recorded and then transferred into the muffle furnace set at 550^{0}C for 4hours. After ashing, the crucibles were cooled to about 105^{0}C in a forced air oven before cooling them further to room temperature in a desiccator. The crucibles and their content were weighed, and weight recorded as percentage ash content and calculated from the relationship below.

$$Ash\ (\%) = \frac{Weight\ of\ crucible + ash - weight\ of\ crucible}{Wt\ of\ sample}\ x\ 100$$

*Crude Fat Determination***:** The flour samples from moisture determination were transferred to a 22×80mm paper thimble. A small ball of cotton wool or glass wool was put into the thimble to prevent loss of sample. Anti-bumping granules were added to a previously dried 250ml round bottom flask and weighed. 150 ml of petroleum spirit was added to the flask and apparatus was assembled. A quick fit condenser was connected to the soxhlet extractor and refluxed for six hours on low heat. The flask was removed and evaporated on a steam bath. The flask with the fat was heated for 30 mins in an oven for

103◦C. The flask and its content were cooled to room temperature in desiccators after which it was weighed and percentage fat was calculated. (AOAC, 1990)

Determination of Crude Fibre: Two gram (2g) of the de-fatted sample was accurately measure into the fibre flask and 100ml of 0.255N H_2SO_4 was added. The mixture was heated for 30min with the heating mantle. The hot mixture was allowed to cool and then filtered through a filter paper. The filtrate obtained was thrown off and the residue was returned to the fibre flask to which 100ml of 0.313N NaOH was added and heated for another 30mins. The mixture was cooled and filtered through a filter paper. The filter paper plus the residue was transferred into a crucible and oven dried at 105^0C for 3hours to drive off moisture. The oven dried crucible containing the filter paper plus was cooled in a desiccators and later weighed to obtain W_1. The crucible with W_1 will then be transferred to the muffle furnace for ashing at 550^0C for 4hours. The crucible containing white or grey ash (free of carbonaceous material) was cooled in the desiccator and weighed to obtain W_2. The difference $W_1 - W_2$ gives the weight of fibre. Then was calculated with the relationship below

$$Crude\ fibre\ (\%) = \frac{Dry\ wt\ of\ residue\ before\ ashing - wt\ of\ residue\ after\ ashing}{Wt\ of\ sample}\ x\ 100$$

Determination of Carbohydrate: The carbohydrate content was determined using the AOAC (2005) method. The content was estimated by difference. % Total carbohydrate = 100-(% water + % protein + % fat + % ash + % crude fibre)

*Mineral Analysis***:** Flour sample (0.5 g) was weighed into a clean ceramic crucible. A blank was prepared with empty crucible. The crucible was placed in a muffle furnace at 500 ^{o}C for 4 hr. The sample was allowed to cool down in the oven after which it was removed carefully. The ashed sample was poured into already labeled 50 mL centrifuge tube. The crucible was rinsed with 5 mL of distilled water into the centrifuge tube. The crucible was rinsed again with 5 mL of aqua regia. This was repeated to make a total volume of 20 mL. The sample was mixed properly and centrifuged (IEC Centra GP8) for 10 min at 301.86 g. The supernatant was decanted into clean vials for mineral determination. The absorbance was read on UV-spectrophotometer (BK-UV1600PC Model) at different wavelength for each mineral element (Ca-422.7 nm, Fe-248.3 nm, Mg-285.2 nm, Na-589 nm and K-766.5 nm) (Novozamsky *et al*., 1983).

$$TTA\ (\%) = \frac{N\ (NaOH) x\ titre\ value\ x\ lactic\ acid\ value\ x\ dilution\ factor}{10}\ x\ 100$$

Where: N = normality of NaOH (0.01), Lactic acid value = 0.09, Dilution factor = 10

*Sensory evaluation***:** Sensory quality attributes of *kunun-zaki* was assessed using 20 panelists. Colour, flavour, taste and general acceptability were based on 9-point Hedonic scale (Iwe, 2000). The rating of the sample ranged from 1 (dislike extremely) to 9 (like extremely).

Statistical Analysis: Data obtained were analysed using One-way Analysis of Variance (ANOVA) using the SPSS (version 17.0) and statistical significance was accepted at 0.05 level of probability. Duncan new multiple test was used to determine difference among means.

*Physicochemical properties of Kunun-zaki***:** pH of the juice was measured using digital pH meter (ELICO L1 614 pH analyser) and expressed as pH units. Total soluble solids (TSS) as % Brix was determined using digital ATAGO refractometer (ATAGO, PAL-Maple Pocket type).

*Titratable acidity***:** It was determined using the AOAC (2000) method. Sample (30 ml) was filtered into a 100ml standard volumetric flask. The filtrate was made up to 100 ml. 10 ml of the filtrate was pipetted into a beaker and 1 drop of phenolphthalein was added. The mixture was then titrated against the standard 0.01 N sodium hydroxide solution until a light pink colour was attained. The reading of the burette was recorded. This was done in duplicates.

RESULTS AND DISCUSSION

Proximate composition of aril of Thaumatococcus danielli: The proximate composition of *Thaumatococcus danielli* aril is shown in Table 1. The aril had low moisture (12.20%). The moisture content of fruit is very important for it shelf life, since the lower the moisture, the better it storage stability (Butt*, et al*., 2004). The ash was 4.79% which indicate high level of minerals in the aril. Fibre, fat, carbohydrate and protein contents were 5.20%, 0.61%, 44.17% and 33.05% respectively. The

aril had high protein content, which made it a suitable substitute for sucrose.

Table 1: Proximate composition of Thaumatococcus *danielli*

Parameter (%)	Aril
Moisture content	12.20±0.02
Ash content	4.79±0.06
Fiber content	5.20±0.09
Fat content	0.61±0.04
Protein content	33.03±0.11
Carbohydrate	44.17±0.20

Mean ± Standard deviation

*Mineral composition of Thaumatococcus dannielli aril***:** Table 2 showed the mineral composition of *Thaumatococcus dannielli* aril. The most abundant mineral in the fruit aril is potassium (190.63ppm) which was in accordance with the report of Oshodi *et al*. (1999) that potassium is the most abundant mineral in Nigeria agricultural products. Potassium and sodium are required to maintain osmotic balance of body fluid pH of the body, regulate muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth (NRC, 1989). Also, values of sodium, calcium, Iron, magnesium were 167.66ppm, 132.96ppm, 21.59ppm and 14.40 ppm. The values of mineral contents are dependent on the following factors season, species, soil types, cultural practices adopted during planting (Nkama, *et al*., 1995).

Table 2: Mineral composition of *Thaumatococcus danielli* aril

Parameter (ppm)	Aril
Potassium	190.63±0.72
Sodium	167.66±1.48
calcium	132.96±1.76
Iron	21.59±0.94
Magnenium	14.40±0.21

Table 3: Physicochemical composition of kunun-zaki sweetened with sucrose

Sample (g)	pH	Total solids	Titratable acidity
2.5	3.90^{d}±0.1	9.15^{c}±0.22	0.93^{c}±0.04
5.0	4.00^{cd}±0.00	10.23^{c}±0.14	0.82^{c}±0.28
7.5	4.15^{bcd}±0.07	10.10^{c}±0.14	0.78^{c}±0.02
10.0	4.40^{bcd}±0.14	10.12^{c}±0.16	0.81^{c}±0.01
12.5	4.45^{b}±0.35	11.85^{b}±0.32	1.39^{a}±0.14
15.0	4.45^{b}±0.70	13.49^{a}±1.29	1.10^{b}±0.14
Control	4.90^{a}±0.14	4.95^{d}±0.11	0.80^{d}±0.01

Values with the same superscript down the column were not significant different (p< 0.05)

Physicochemical composition of kunun-zaki sweetened with Thaumatococcus dannielli: Physicochemical composition of Kunun-zaki sweetened with *Thaumatococcus dannielli* are presented in Table 4. pH contents ranged from 3.51 to 4.90. There were slight increases in the pH of kunun-zaki with increase in *Thaumatococcus dannielli* addition. The control was significantly different ($p < 0.05$) from other samples in pH.According to Kaanane, *et al*. (1998) the minimal change in pH can be explained by relationship existing between pH and free acid content. The high pH of a food is used as an indicator of bacterial spoilage i.e the food with high pH is more susceptible to microbial spoilage. At high concentration (12.5 and 15.0 g), the total solids were 7.43 and 7.23 % respectively. There were no significant differences ($p < 0.05$) in these values. Addition of *Thaumatococcus danielli* to *kunun-zaki* increased the total solids of *kunun-zaki.* Titratable acidity ranged from 0.74-0.85 %. Titratable acidity increased with increase in *Thaumatococcus danielli* concentration but there were no significant differences ($p < 0.05$) among the *kunun-zaki*.

Mean ± Standard deviation

Physicochemical composition of Thaumatococcus dannielli aril: Physicochemical composition of *Kunun-zaki* with sucrose is shown in Table 3.pH ranged from 3.90-4.90. The pH of the control was significantly different ($p < 0.05$) from other *kunun-zaki* samples. Increase in sucrose concentration reduced the pH of kunun-zaki. The pH of the samples increased as the values of sucrose increased, this may be as a result of the fact that, the samples were freshly prepared and the sugar in samples had not broken down as a result of fermentation. No significant differences ($p < 0.05$) in pH values of sucrose concentration from 2.5 to 10.0 g. pH is a measure of activity of the hydrogen ion and it measures the hydrogen ion concentration. pH is an index of the level of acidity or alkalinity of a sample and this has great effect on the storage period (Dandago*, et al*., 2004).This is in line with the report of Deans and Ritchie (2005) with pH of 4.67 for pawpaw juice. The titratable acidity of samples with 2.5g, 5.0g, 7.5g, and 10.0g values of sucrose were significantly different ($p<0.05$) from others, while samples with 12.5g, 15.0g were not significant different ($p<0.05$). The total solids followed the same trend as titratable acidity in all the samples. There were no significant differences ($p<0.05$) in samples 12.5g and 15.0g sucrose. The control sample was significantly different ($p<0.05$) from other samples. Total solid is the amount of dry material remaining after all the water is evaporated.

Table 4: Physicochemical composition of kunun-zaki with *Thaumatococcus danielli*

Sample (g)	pH	Totalsolids	Titratable acidity
2.5	3.51^{d}±0.13	5.33^{de}±0.16	0.74^{a}±0.29
5.0	3.70^{d}±0.01	5.81^{cd}±0.12	0.77^{a}±0.10
7.5	3.57^{d}±0.07	6.67^{b}±0.14	0.82^{a}±0.01
10.0	3.75^{d}±0.13	6.56^{b}±0.15	0.85^{a}±0.01
12.5	3.97^{c}±0.42	7.43^{a}±0.15	0.85^{a}±0.04
15.0	3.96^{c}±0.14	7.23^{a}±0.66	0.83^{a}±0.07
Control	4.90^{a}±0.14	4.95^{c}±0.11	0.80^{a}±0.01

Values with the same superscript down the column were not significant different (p< 0.05)

Comparative study on the physicochemical properties of *kunun zaki* with sucrose and *Thaumatococcus danielli* are

shown in Table 5.Control had higher pH value which was significantly different ($p<0.05$) from other samples. This indicated that the control had higher tendency to deteriorate than other *kunun-zaki. Kunun-zaki* with 15.0g sucrose had higher total solid while that with 12.5g sucrose concentrations had higher titratable acidity which were significantly different ($p< 0.05$) from other samples.

Table 5: Comparative study on the physicochemical properties of kunun-zaki with sucrose and *Thaumatococcus daninelli* aril

Sample	Concentration (g)	pH	Total solids	Titratable acidity
Sucrose	2.5	$3.90^{cd}\pm0.10$	$9.15^{a}\pm0.22$	$0.93^{c}\pm0.04$
	5.0	$4.00^{cd}\pm0.00$	$10.23^{c}\pm0.14$	$0.82^{cd}\pm0.28$
	7.5	$4.15^{bc}\pm0.07$	$10.10^{cd}\pm0.14$	$0.78^{d}\pm0.02$
	10.0	$4.40^{b}\pm0.14$	$10.12^{cd}\pm0.16$	$0.81^{cd}\pm0.01$
	12.5	$4.40^{b}\pm0.35$	$11.85^{b}\pm0.32$	$1.39^{a}\pm0.14$
	15.0	$4.45^{b}\pm0.70$	$13.49^{a}\pm1.29$	$1.10^{b}\pm0.14$
Thaumatococcus danielli	2.5	$3.51^{e}\pm0.13$	$5.33^{gh}\pm0.16$	$0.74^{d}\pm0.29$
	5.0	$3.70^{cd}\pm0.01$	$5.81^{fgh}\pm0.12$	$0.77^{d}\pm0.10$
	7.5	$3.57^{e}\pm0.07$	$6.67^{ef}\pm0.14$	$0.82^{cd}\pm0.01$
	10.0	$3.75^{cd}\pm0.13$	$6.56^{ef}\pm0.15$	$0.85^{cd}\pm0.01$
	12.5	$3.97^{cd}\pm0.14$	$7.43^{e}\pm0.15$	$0.85^{cd}\pm0.04$
	15.0	$3.96^{cd}\pm0.14$	$7.23^{e}\pm0.66$	$0.83^{cd}0.07$
control		$4.90^{a}\pm0.14$	$4.95^{h}\pm0.11$	$0.80^{d}\pm0.01$

Values with the same superscript down the column were not significant different ($p< 0.05$)

*The Sensory Evaluation of Kunun-zaki with Thaumatococcus danielli aril and Sucrose***:** The mean scores for sensory characteristics of *kunun-zaki* with *Thaumatococcus danielli* and sucrose are presented in Table 6. The sensory data revealed that there were no significant differences ($p<0.05$) in the colour and aroma of the *kunun-zaki.* Taste of *kunun-zaki* with sucrose at 7.5 g concentration was preferred among the samples. Increase in *Thaumatococcus danielli* aril improved the taste of *kununzaki* but the tastes of *kunun-zaki* at higher sucrose concentration were not acceptable. Overall acceptability signified that *kunun-zaki* with low sucrose concentration (7.5g) were more acceptable than other samples followed by *kunun-zaki* with 2.5 g sucrose concentration and 15.0 g *Thaumatococcus danielli* aril. This showed that the higher the concentration of *Thaumatococcus danielli* aril, the higher the acceptability.

Table 6: Sensory Evaluation of kunun-zaki with *Thaumatococcus danielli*

Sample	Colour	Taste	Aroma	Overall Acceptability
2.5 KZT	$2.22^{a}\pm1.30$	$2.00^{h}\pm0.00$	$4.00^{a}\pm0.87$	$2.56^{g}\pm0.88$
7.5 KZT	$2.22^{a}\pm1.30$	$3.56^{f}\pm0.53$	$4.00^{a}\pm0.87$	$2.78^{f}\pm0.83$
12.5KZT	$2.22^{a}\pm1.30$	$4.60^{e}\pm0.88$	$4.00^{a}\pm0.87$	$4.44^{d}\pm0.88$
15.0KZT	$2.22^{a}\pm1.30$	$6.11^{c}\pm0.61$	$4.00^{a}\pm0.87$	$5.11^{c}\pm2.32$
2.5KZS	$2.22^{a}\pm1.30$	$7.11^{b}\pm0.61$	$4.00^{a}\pm0.87$	$7.00^{b}\pm2.65$
7.5KZS	$2.22^{a}\pm1.30$	$8.89^{a}\pm0.33$	$4.00^{a}\pm0.87$	$7.33^{b}\pm2.78$
12.5KZS	$2.22^{a}\pm1.30$	$2.89^{g}\pm0.33$	$4.00^{a}\pm0.87$	$2.56^{g}\pm0.53$
15.0KZS	$2.22^{a}\pm1.30$	$4.78^{e}\pm1.40$	$4.00^{a}\pm0.87$	$3.22^{e}\pm0.83$
Control KZ	$2.22^{a}\pm1.30$	$5.45^{d}\pm0.88$	$4.00^{a}\pm0.87$	$4.56^{d}\pm0.73$

Values with the same superscript down the column were not significant different ($p< 0.05$)
KZT= kunun-zaki with *Thaumatococcus danielli*, KZS= kunun-zaki with sucrose and control = KZ

Conclusions: The result from proximate composition revealed that *Thaumatococcus danielli* aril has high protein which could be used as substitute for synthetic sugar since it is of calorie free. Also this fruit is one of the underutilized fruits which when processed can replace other sweeteners which can pose health challenge. Addition of more *Thaumatococcus danielli* aril to *kunun-zaki* could improve the acceptability of the product.

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