Madras Agric. J., 2023; https://doi.org/10.29321/MAJ.10.000762

RESEARCH ARTICLE

Formulation and Optimization of Functional Milk Beverage using D – optimal Mixture Design

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Received: 24 February 2023

Revised: 10 February 2023

Revised: 17 February 2023

Accepted: 22 March 2023

ABSTRACT

Functional foods are in great demand worldwide because of their many health benefits. As milk is a nutrient-dense diet that comprises all vital nutrients, it can be utilized as the primary base to produce a variety of functional foods. Ginger, cinnamon and pumpkin possess tremendous nutritional and therapeutic properties such as stimulating the digestive system, lowering blood cholesterol levels and exhibiting antioxidant,anti-diabetic, antibacterial, antiviral and anti-carcinogenic activities, preventing oxidative stress-related diseases and in the ageing process. In this study, aqueous cinnamon and ginger extracts and pumpkin pulp were made and their functional properties like antioxidant activity and total phenolic content have been evaluated. Functional milk beverage was made using milk, cinnamon aqueous extract, ginger aqueous extract and yellow pumpkin pulp. The level of inclusion of these components was optimized using D-optimal mixture design (Design Expert Software) with regard to sensory attributes.The optimized level of functional milk beverage was chosen based on the desirability of the design and the range assigned to each response. The level of incorporation of milk, pumpkin pulp, aqueous cinnamon and ginger extracts were optimized at 40%, 10%, 20%, and 30% respectively. The antioxidant activity of the cinnamon aqueous extract, ginger aqueous extract, pumpkin pulp and optimized functional milk beverage was found to be 69.95 ± 0.07 %, 56.45 ± 0.08 %, 25.80 ± 0.10 and 60.53 ± 0.03 % respectively. The total phenolic content of cinnamon aqueous extract, ginger aqueous extract, pumpkin pulp and optimized functional milk beverage were 450.88 ± 0.03 mgGAE/100g, 106.93 ± 0.02 mgGAE/100g, 15.59 ± 0.09 mgGAE/100g and 280.63 ± 0.02 mgGAE/100g respectively.

Keywords: *Functional milk beverage; Ginger; Cinnamon; Pumpkin; Sensory; Antioxidant activity; Phenolic content.*

INTRODUCTION

Functional foods have integrated themselves into our dietary patterns. Functional food is "a food stuff that provides a health benefit beyond basic nutrition, demonstrating specific health or medical benefits, including the prevention and treatment of disease," by the Food and Agriculture Organization, (FAO). When consumed, they should provide specific therapeutic benefits such as disease prevention and rehabilitation, management and control of physical and mental health and reduced ageing. The Indian consumer of today is more receptive to new concepts, concerned with nutrition and health and driven by a need for higher-quality, more functional food products. According to statistics from 2020, the global market for functional foods brought in about $247.89 billion in sales in 2017 and was anticipated to reach

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$319.93 billion by 2022 (Purwaningsih *et al*., 2021).

People worldwide rely heavily on milk and other dairy products in their diets. Milk contains valuable nutrients, including protein, fat, carbohydrate, vitamins and minerals. Due to the intrinsic nutritional composition, sensory appeal and health-promoting qualities, milk can be used as the base for functional beverages employed in the development of nutraceuticals and value-added food products.

Herbs, spices and vegetables contain a broad range of bioactive substances, phytochemicals, phenolic compounds that exhibit integrated antioxidant, anti-inflammatory properties and have various functional and therapeutic properties. Because of their beneficial impact on humans, they can be used as potential ingredients in functional foods. (Viuda-Martos *et al*., 2010; Serafini and Peluso, 2016 and Wanna, 2019).

A major issue affecting the entire world in 2019 is the coronavirus disease epidemic (COVID -19), which is caused by severe acute respiratory syndrome. Many scientists and researchers had challenges due to the intricacy and difficulty of developing new medications, as well as the severity of the pandemic. They suggested using low-cost, effective home remedies and immune system boosters like consuming spices and herbs, as these are some of the most significant ways to manage the prevailing pandemic crisis. (Yakhchali *et al*.,2021).

Ginger, cinnamon and yellow pumpkin have a significant nutritional profile and therapeutic potential because they contain various bioactive, phenolic and non-phenolic constituents. The main bioactive component in ginger includes gingerols, shogaols and paradols, cinnamon contains polyphenols and cinnamaldehyde and yellow pumpkin has a richness of bioactive compounds such as carotene, phenolics, flavonoids, and terpenoids. Ginger, cinnamon, and yellow pumpkin exhibit various functional and therapeutic properties comprising anti-microbial, anti-fungal, antiviral, antioxidant, anti-tumor, anti-diabetic, lowering blood pressure, lowering cholesterol and lipids and gastro-protective properties. The significant role of ginger, cinnamon and yellow pumpkin is managing and treating several illnesses, including oxidative stress, diabetes, cancer, arthritis, gout, gastric ulcer, hypercholesterolemia, pain and bacterial or viral infection, and can be used as a postoperative antiemetic. People with type II diabetes benefit from cinnamon because it stimulates their ability to react to insulin, which normalizes blood sugar levels. (Djutin, 1991; Asgary *et al*., 2011, Zadeh and Kor, 2014, Gunathilake and Rupasinghe, 2015; Perez Gutierrez, 2016; Oaron *et al*.,2017, Ribeiro-Santos *et al*.,2017, Goel and Mishra,2020).

Functional milk beverage is a flavoured milk made with milk, ginger, cinnamon and pumpkin pulp. The functional milk beverage has health benefits such as antioxidant activity, improved bone health, reduced risk of cardiovascular disease, cancer, ulcer, type 2 diabetes and improved immunity and many more. D-Optimal mixture design provided by Design Expert Software is a very efficient tool for optimizing ingredients to produce products with desirable attributes (Awang *et al*., 2008). The formulation and optimization of the three ingredients - ginger, cinnamon and pumpkin pulp, in the development of functional beverages using Design Expert Software is described in this research paper, along with selecting the optimal beverage based on sensory attributes satisfying consumer's sensory acceptability.

The present study is conceived with the idea to develop a functional milk beverage incorporated with spice, herb, and vegetable pulp to cater to the growing needs of customers who look for innovative functional foods.

MATERIAL AND METHODS

Raw materials

Toned milk (3% Fat and 8.5% SNF) is generally used for the preparation of flavoured milk. It is found ideal for the preparation of milk beverages and hence toned milk (procured from Aavin, {TCMPF}, Tamil Nadu) was used in this study. Dry ginger, cinnamon bark and yellow pumpkin were purchased from the local market.

METHODS

Cinnamon Aqueous Extract (CAE)

Cinnamon aqueous extract was prepared as per the method of Aneja *et al*. (2009) and Sana *et al*. (2019) with some modifications. Cinnamon bark was ground into powder using a mixer grinder and sieved twice to obtain a fine powder. 10 g of Cinnamon powder was added to 100 ml of distilled water. The content was placed on a magnetic stirrer for 60° C for 60 min for uniform and continuous

mixing of the aqueous mixture. The aqueous mixture was allowed to cool and filtered using Whatman No.1 filter paper and then stored in an amber-coloured bottle in the refrigerator till further use.

Ginger Aqueous Extract (GAE)

Ginger Aqueous Extract was prepared as per the method of Tohma *et al*. (2017) with some modifications. Dry ginger was ground into powder using a mixer grinder and sieved twice to obtain a fine powder. 25 g of dry ginger powder was dissolved in 100 ml of distilled water. The content was placed on magnetic stirrer for 60 min for constant stirring of the mixture and it was filtered first using a muslin cloth. The second filtration is done using Whatman No.1 filter paper and then stored in an amber-coloured bottle in the refrigerator till further use.

Pumpkin pulp

Pumpkin outer skin was peeled, cut into small pieces, washed thoroughly and added to boiling water and fully cooked. After cooling, it was pureed using a household mixer grinder, filtered and used during beverage preparation at the required level (Dini *et al*., 2013).

Experimental design for the development of beverage formulations

Mixture design provided by Design Expert Software (Version 12) is a statistical tool opted in this study for the optimization of ingredients to produce products with desirable attributes. It enables understanding of the relationships and interactions between the components. It determines and simultaneously resolves multivariate equations that can be represented graphically using quantitative data from appropriate experimental designs.

D-optimal mixture design was chosen to optimize the functional milk beverage made from milk, pumpkin pulp, cinnamon aqueous extract and ginger aqueous extract. The level of milk is kept constant (40%). The level of incorporation of pumpkin pulp, cinnamon aqueous extract and ginger aqueous extract were considered independent variables. The sensory attributes viz., colour, flavour, taste, mouthfeel and overall acceptability were considered as dependent variables. Based on preliminary trials, the independent variables were assigned minimum and maximum values as shown in Table 1. The number of runs created in design expert software was 16, as given in Table 2.

These 16 beverage combinations were blended and provided for the sensory assessment using a 9-point hedonic scale. The responses were based on the sensory attributes of colour, flavour, taste, mouthfeel and overall acceptability. The average responses of the panellists were entered into design expert software. Using design expert software, the responses of the panellists were optimized through numerical and graphical representation. Based on the desirability of the design and the range assigned to each response, the optimum beverage levels were selected. The optimized beverage was developed and then subjected to further processing.

Table1. Experimental ranges and levels of independent variables used in D-optimal mixture design for the formulation of functional milk beverage

S.No.	Components	Low-level	High-level
1	Pulp	5	10
2	Cinnamon	15	25
3	Ginger	25	35

Table2. Design of experiment for functional beverage formulae proposed by Design-Expert Software

S.No.	Pumpkin Pulp	Cinnamon	Ginger
1	10	25	25
2	7.5	25	27.5
3	7.5	19.37	33.12
4	10	20	30
5	7.5	17.5	35
6	5	20	35
7	6.25	23.12	30.62
8	10	15	35
9	8.75	23.12	28.12
10	10	20	30
11	8.75	18.12	33.12
12	5	25	30
13	10	15	35
14	10	25	25
15	5	25	30
16	5	20	35

Sensory evaluation

Sensory evaluation of prepared functional milk beverage using a 9 - point Hedonic scale (Larmond, 1977) was carried out by a panel of judges. All the samples were appropriately coded before being subjected to sensory evaluation. The sensory score obtained was fed into design expert software to obtain the optimal solution.

Hedonic ratings were done by panellists who evaluated colour and appearance, flavour, taste, mouth feel and overall attributes of the functional milk beverage on a 9 - point scale: (1) dislike extremely, (2) dislike very much, (3) dislike moderately, (4) dislike slightly, (5) neither like nor dislike (neutral), (6) like slightly, (7) like moderately, (8) like very much, (9) like extremely. Responses were then analysed by *Design Expert®* 7 to determine the optimum beverage formula.

Determination of functional properties of CAE, GAE, pumpkin pulp and optimized functional milk beverage.

Total phenolic content

The total phenols were determined using Folin-Ciocalteu method as described by Singleton *et al.* (1999) with some modifications.

Antioxidant Activity

Antioxidant activity was determined by 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) method as described by Shimada *et al.* (1992) with some modifications.

RESULTS AND DISCUSSION

Beverage standardization– D optimal mixture design

The milk, pumpkin pulp, cinnamon aqueous extract and ginger aqueous extract were blended and given for sensory evaluation using a 9 – point hedonic scale. The sensory attributes viz., colour and apperance, flavour, taste, mouth feel and overall accept ability were considered as responses. The average of responses obtained from the panellists was entered in design expert software (D – Optimal mixture design). The average responses of the panellists are given in Table 3.

The average responses of the panellists was entered and optimized by numerical and graphical representation using design expert software (D–optimal mixture design). The software generated polynomial equations for sensory attributes (colour and appearance, flavour, taste, mouthfeel and overall acceptability) score as a function of the combinations of the functional milk beverage. The polynomial equation of each response revealed interaction effects among ingredients. Based on D - optimal mixture design analysis, a cubic model of sensory attributes was derived that fit the data with statistical significance.

A significant cubic model and a non-significant lack of fit were both shown in the analysis of the variance table given in Table 4. The p-value indicates that the model was significant and shows the variation in responses. F-value shows lack of fit is not significant, indicating that the model fits the actual data within the limit. The coefficient of regression (R^2) near one indicates that the model was good.

The three-dimensional graph (3D) for responses of sensory parameters (colour and appearance, flavour, taste, mouthfeel and overall acceptability) is prescribed in Figure 1 - 5. The relationship between the utilization of milk, pumpkin pulp, cinnamon aqueous extract, and ginger aqueous extract and the resulting sensory response value is illustrated in the residual plot graph in Figures 1 – 5. The red portion of the graph indicates the highest response for colour and appearance, flavour, taste, mouthfeel and overall acceptability are 8.7, 8.6, 8.3, 8.4 and 8.5 respectively, with the criteria of liking very much. The combination of milk, pumpkin pulp, CAE and GAE, 40%, 10%, 20%, and 30%, respectively fall in the red portion of the graph. While the blue portion shows the lowest response for colour and appearance, flavour, taste, mouthfeel and overall acceptability are 5.8, 5.6, 5.7, 5.4 and 5.6 respectively with a mild dislike set of criteria. The combination of milk, pumpkin pulp, CAE, and GAE, 40%, 10%, 15%, and 35%, respectively fall in this blue portion of the graph.

The optimized levels of functional milk beverage were chosen based on the desirability of the design and range assigned to each of the responses. To achieve the optimum combination, each individual factor and response was ranked according to importance (Table 5). The sensory parameters Colour and appearance, flavour, taste, mouthfeel and overall acceptability were considered to be of maximum importance because of the significant influences of these factors on both functionality and sensory acceptance, while the components were considered to be in range importance. The optimized level of pumpkin pulp, cinnamon, ginger and milk was used to prepare a functional beverage. The optimum solutions of components and responses are given in Table 6.

To optimize the level of functional milk beverage with high desirability and to maximize the sensory responses, design expert software was used as a tool, which allows the optimum level of milk, pumpkin pulp, CAE and GAE with a minimal number of runs. The goal of optimization of functional milk beverage was to maximize the sensory scores. From the D-Optimal mixture design, 4th run was selected based on the desirability and it was used to prepare the functional milk beverage. The results of the present study correlated with Jayachandran *et al*. (2015), who stated that numerical optimization was used to determine the best sensory score value for each sensory feature. The result coincided with the findings of Andriansyah *et al*. (2022) who optimized the herbal tea drink formula using D-Optimal mixture design. The sensory score obtained in his study with criteria of liking was colour attributes 3.63, taste 3.973, smell 3.963 and overall acceptability of 3.934 and desirability (0.604). Both desirability and sensory scores were used to compute overall acceptance. All the created models had r^2 and adjusted r^2 values greater than 0.90, indicating that the response surfaces were well-fitted with the experimental data.

Functional Properties of CAE, GAE, pumpkin pulp and optimized beverage

The functional properties such as antioxidant activity and total phenolic content of cinnamon aqueous extract, ginger aqueous extract, pumpkin pulp and optimized beverage were determined and mentioned in Table 7. It was found that cinnamon aqueous extract has the highest antioxidant activity and total phenolic content compared to ginger aqueous extract, pumpkin pulp and optimized functional milk beverage. The current finding was similar to those reported by Sana *et al*. (2019) who evaluated the phenolic profile and antioxidant of cinnamon and turmeric extracts. The TPC of cinnamon aqueous extract, according to their study, was 548.87 mg GAE/100g which is in close accordance with the results of the present study. Vidanagamage *et al*. (2016) studied the effect of cinnamon extract on butter; they found that the antioxidant activity of the extract was 81.33 ± 0.002% which is in close agreement with the present experimental values. Tohma *et al*. (2016) determined the phenolic and antioxidant acticity of ginger extract and it was found to be 52.8µg/mg and 38.9% respectively

And the result coincides with their findings. According to Dini *et al*. (2013) the antioxidant activity and total phenolic content of pumpkin pulp were 374.66 ± 2.7 µmol/10 g and 53.77 ± 1.8 (GAE)/10 g, which agrees with the obtained result. Guar *et al*. (2019) developed herbal milk using tulsi juice, ginger juice and turmeric powder and determined the antioxidant activity and phenolic content of herbal milk which was found to be 50.14% and 96.25 mg GAE/100g. The result obtained was higher than that of the findings of Guar *et al*. (2019), due to the cinnamon aqueous extract, which has tremendous phenolic and antioxidant activity and is responsible for the higher functional properties of the developed beverage.

Table 5. Importance criteria for functional milk beverage

Component/ Responses	Goal	Minimum	Maximum
A: Pumpkin pulp	Is in range	5	10
B: Cinnamon	Is in range	15	25
C: Ginger	Is in range	25	35
Colour and Appearance	Maximize	5.8	8.7
Flavour	Maximize	5.6	8.6
Taste	Maximize	5.7	8.3
Mouthfeel	Maximize	5.4	8.4
Overall Acceptability	Maximize	5.6	8.5

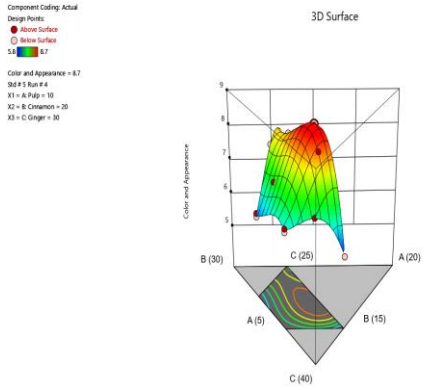

Figure1. 3D Graph for colour and appearance

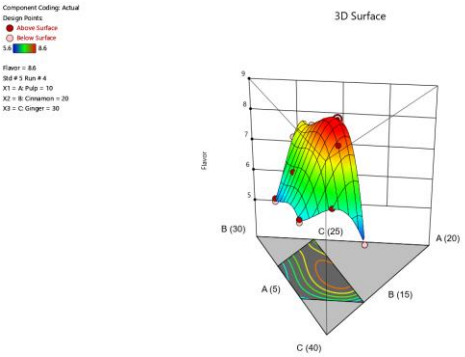

Figure 2. 3D Graph for Flavour

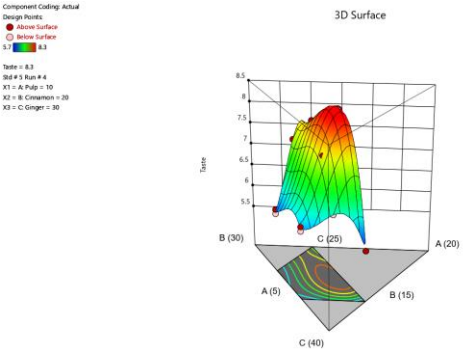

Figure 3. 3D Graph for taste

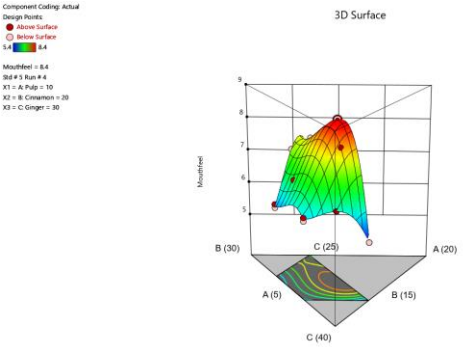

Figure 4. 3D Graph for mouthfeel

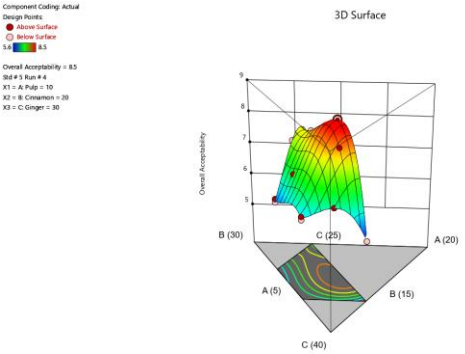

Figure 5. 3D Graph for overall acceptability

Table 3. Experimental values based on responses

Run	Pumpkin pulp	Cinnamon extract	Ginger extract	Colour and appearance	Flavour	Taste	Mouth feel	Overall acceptability
1	10	25	25	7.5	7.3	7.1	7.1	7.2
2	7.5	25	27.5	7.8	7.6	7.4	7.3	7.5
3	7.5	19.375	33.125	8	7.8	7.6	7.5	7.7
4	10	20	30	8.7	8.6	8.3	8.4	8.5
5	7.5	17.5	35	6.9	6.7	6.5	6.3	6.6
6	5	20	35	6.6	6.3	6.2	6.1	6.3
7	6.25	23.125	30.625	7.2	7	6.8	6.7	6.9
8	10	15	35	5.8	5.6	5.7	5.4	5.6
9	8.75	23.125	28.125	8.2	8.1	7.9	7.7	7.9
10	10	20	30	8.6	8.5	8.2	8.3	8.4
11	8.75	18.125	33.125	8.4	8.3	8	7.9	8.1
12	5	25	30	6.1	5.9	5.9	5.8	5.9
13	10	15	35	5.8	5.6	5.7	5.4	5.6
14	10	25	25	7.4	7.2	7	7	7.1
15	5	25	30	6.2	6	6	5.9	6
16	5	20	35	6.5	6.2	6.1	6	6.2

Table 4. Analysis of variance of cubic model for the responses

Responses	Model	P value	F value	Lack of fit	P value	F value	R^2
Colour and appearance	Significant	<0.0001	416.22	Not Significant	0.3790	0.9306	0.9984
Flavour	Significant	<0.0001	367.87	Not Significant	0.1892	2.31	0.9982
Taste	Significant	<0.0001	420.66	Not Significant	0.9911	0.0001	0.9984
Mouthfeel	Significant	<0.0001	492.20	Not Significant	0.9187	0.0115	0.9986
Overall acceptability	Significant	<0.0001	452.49	Not Significant	0.6023	0.3089	0.9985

Table 6. Optimum solution of components and responses

Pulp	Cinnamon	Ginger	Colour & Appearance	Flavour	Taste	Mouthfeel	Overall Acceptability	Desirability	
9.560	**19.309**	**31.131**	**8.856**	**8.765**	**8.469**	**8.492**	**8.615**	**0.994**	**Selected**
10.00	18.975	31.025	8.770	8.655	8.348	8.484	8.570	0.991	

Table 7. Functional properties of CAE, GAE, pumpkin pulp and optimized beverage

Functional properties	Cinnamon aqueous extract	Ginger aqueous extract	Pumpkin pulp	Optimized functional milk beverage
Antioxidant Activity (%)	69.95±0.07	56.45±0.08	25.80 ± 0.10	60.53±0.03
Total Phenolic Content (mg GAE/100g)	450.88±0.03	106.93±0.02	15.59 ± 0.09	280.63±0.02

@ Average of six trails

Conclusion

According to the outcomes of the current investigation, the D-Optimal mixture design was found to be the best for optimizing ingredients to develop a functional milk beverage with limited runs and desirable sensory attributes. The aqueous extract of cinnamon and ginger was found to have functional benefits due to its antioxidant activity and phenolic content. Pumpkin pulp, cinnamon and ginger were incorporated into the milk to give it a distinctive flavour and enhance the product's sensory appeal and acceptability. The optimized level obtained from the D-Optimal mixture design was milk, pumpkin pulp, cinnamon aqueous extract and ginger aqueous extract to be incorporated at 40%, 10%, 20% and 30%, respectively with best sensory score of colour and appearance (8.856), flavour (8.765), taste (8.469), mouthfeel (8.492) and overall acceptability (8.615) with good desirability of 0.994. The antioxidant activity and total phenolic content of cinnamon aqueous extract, ginger aqueous extract, pumpkin pulp and optimized beverage formulation were found to be 69.95 ± 0.07%, 56.45 ± 0.08%, 25.80 ± 0.10%, 60.53 ± 0.03% and were 450.88 ± 0.03 mg GAE/100g, 106.93 ± 0.02 mg GAE/100g, 15.59 ± 0.09 mg GAE/100g, 280.63 ± 0.02 mg GAE/100g respectively. Since the developed beverage is rich in antioxidant activity and phenolic content, this will provide numerous health benefits, such as anti-microbial, anti-inflammatory, cardio-protective, anti-carcinogenic and anti pyretic to the consumer.

Funding and Acknowledgment

No funding

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

We ensure that we have written and submitted only entirely original works, and if we have used the work and/or words of others, that has been appropriately cited.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Author contributors

MohanakKaviya I, Dr. B. Murugan, Dr. Rita Narayanan, Dr. M. Esther Magdalene Sharon contributed to optimize the functional milk beverage and study their functional properties.

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