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Effect of banana flour on composition and shelf life of concentrated whey incorporated bread

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Abstract
In an attempt to use banana flour for preparation of bread, banana flour was added with wheat flour in proportions of 10%, 20%, 30%, 40%, 50% and 60%. This mixture was converted to dough by adding concentrated whey (12% TS) to manufacture bread which was evaluated for sensory, physico- chemical and textural properties. The study indicated that concentrated paneer whey can be used to replace 100 % water in preparation of bread. Amongst the different combinations of wheat flour and banana flour, bread prepared from 60% wheat flour and 40 % banana flour was found to be acceptable for sensory and textural properties. Control bread and bread prepared from 60% WF and 40 % BF was analyzed for proximate, physico-chemical and microbiological properties. The optimized bread had higher protein (9.70±0.10%), dietary fiber (5.20±0.12%), resistant starch (6.81±0.14%) and ash (3.33±3.10%). The combination 50% Co_2: 50% N_2 depicts higher acceptability under MAP during storage up to 20 days. Results revealed BF could be added up to 40 % to prepare bread of acceptable quality and thereby utilize banana flour and concentrated whey to increase the nutritional value of bread.

Keywords: Bread, paneer whey, banana flour, sensory evaluation, modified atmosphere packaging

Introduction

Whey is a major by-product of dairy industry during manufacture of products like cheese, Paneer, Channa, Chakka and casein. It is a rich source of lactose (4-5%), minerals (0.60%) and whey proteins (0.7-0.8%) (Nair & Thompkinson, 2007) [20]. Whey proteins have been considered as superior to most of the other proteins such as egg, beef, casein and soy proteins in nutritive values. Whey is also rich in micronutrients like milk salts, water soluble vitamins, peptides, immunoglobulins and lactoferrin (IDF, bulletin, 2003) [14]. The world whey production is over 160 million tons per year, showing a 1-2 per cent annual growth rate (Bozanice *et al.*, 2014) [7]. Most of the whey drained off causing pollution to the environment and loss of valuable milk solids which can be better utilized as a source of nutrients. Processing of whey is one of the means to tackle these problems. Now a day's whey could be processed and used in various types of food products. The Indian bakery industry is the largest sectors of the processed food industry accounting for value output of 210 billion rupees (Annonymous, 2010) [7]. There is an immense scope of whey utilization in the manufacture of bakery products. Bakery goods are the products rich in carbohydrates but poor in proteins. In addition to it, whey proteins contain high level of essential amino acids and considered as a source of high quality proteins. In this context the present study was initiated to utilize concentrated whey in the production of bread.

Banana is one of the world's most important food crops and they are grown in large quantities in both tropical and subtropical countries. Banana fruit is rich in minerals (potassium, magnesium and phosphorus), dietary fiber, and various antioxidants, such as vitamin A, vitamin C, vitamin E, and β-carotene (Kanazawa and Sakakibara, 2000) [15]. Drying of unripe green bananas and transforming them into banana flour is one of the novel approaches of its utilisation. The resistant starch present in green bananas is available to the bacterial microflora in the large intestine and results in some health benefits like reduction in blood glucose level. But during ripening this starch reserve decreases due to enzymatic action. So the unripe banana flour was used in the preparation of bread. Wheat flour is one of the most widely used ingredients for the manufacture of bread, which is the most widely consumed staple food. Unfortunately, life- style diseases are very common in the urban areas of India. Since bread is consumed by most of the peoples of all age groups every day, with the addition of banana flour, it could be a good vehicle with improved nutritive value. Hence the objective of this study was to evaluate the effect of partial substitution of wheat flour with unripe banana flour on physico-chemical, textural and shelf life of high fiber bread.

Materials and Methods
Materials
Paneer whey was obtained from the Pilot plant of Institute of Agricultural Sciences. Paneer whey was obtained by the coagulation of milk using citric acid. Whole wheat flour (of commercial grade), banana flour (BF), sugar, salt, fat and yeast were used as ingredients in the preparation of bread. All the chemicals used in the study were purchased from M/s Hi-Media (P) Ltd., Mumbai.

Methods
Processing of whey

Whey obtained from paneer production was concentrated in a single effect evaporator to obtain concentration of approximately 25% TS before using in the preparation of bread dough for baking (Chauhan *et al.*, 2016) [9]. Water used for dough making was totally replaced with this concentrated whey for preparation of bread.

Preparation of banana flour

Fresh good quality banana at unripe stage were procured from the local market of Varanasi. Banana flour was prepared by method of (Sajlate *et al*., 2006) [23]. After washing, banana was dipped in hot water (70ºC) for 10 minutes until they become tender. After cooling under tap water (23ºC) they were peeled and sliced (3-4 mm thick) and the slices were dipped in 0.2% (w/v) citric acid solution for 10 min to reduce the enzymatic browning. After draining, slices were dried using tray drier (Khera Instrument Pvt. Ltd.) at 60ºC, grinded and sieved (250 µ) to obtain banana flour.

Preparation of BF bread incorporated with concentrated whey

The whole wheat flour (WF) (120 g) and BF (80 g) were mixed and concentrated whey (98.78 mL) was added to replace 100% water in the preparation of bread loaf (200 g). The final optimized procedure of BF supplemented bread is shown in Figure 1.

Chemical analysis

The moisture, ash and fat content in bread were determined as described in (AOAC, 2005) [5]. The total carbohydrates were adjusted by difference method. The total protein content was estimated by micro- kjeldhal method. The dietary fiber was determined by the method recommended by (AOAC, 1999) and resistant starch was measured by the method proposed by Goni *et al.,* (1996) [13].

Microbiological analysis

Two different combinations of gases i.e. 98% N_2 and 50% N_2: 50% CO_2 were used to pack the bread samples and then analysed for fungal count, coliform count and total plate count at the interval of five days up to a period of 20 days.

Texture evaluation of bread

The texture of bread was assessed by texture profile analyser (Make: TA-XT plus, Stable Micro System, UK) by the method of Dvorakova *et al*., (2012) [12].

Sensory evaluation

The sensory evaluation was done by semi-trained panel at Banaras Hindu University, Varanasi by using 9-point hedonic scale for colour, taste, crumb texture and overall acceptability.

Determination of total phenolic content and free radical scavenging activity

Total phenolic content determination is based on a chemical reduction of reagent, which is a mixture of tungsten and molybdenum oxides and was determined by Folin-Ciocalteau (FC) colorimetry (Matthaus, 2002) [19]. DPPH (2,-2-Diphenyl-1-picryl hydrazyl) free radical scavenging activity in bread was evaluated by the method of Brand-Willaims *et al*., (1995) [8]. 0.1mL of methanolic extract of bread was mixed with 2.9 mL of 0.1mM Methanolic DPPH solution. Control was prepared by mixing 0.1 mL of methanol with 2.9 mL of 0.1mM methanolic DPPH solution and methanol was used as blank. The samples were kept in the dark room for 30 minutes after which absorbance was recorded at 517 nm. The DPPH free radical scavenging activity was computed using the following formula.

$$\text{\% DPPH free radical scavenging activity} = \frac{\text{Control Absorbance} - \text{Sample absorbance)}}{\text{Blank absorbance}} \times 100$$

Shelf -life Evaluation

The bread samples were packaged in low density polyethylene (LDPE) pouches using modified atmosphere packaging (MAP) equipment (Manufacture, PBI, Dansensor A/S Ringsted, Denmark) by using two different gas compositions *viz*., 98% N_2 and 50% N_2: 50% CO_2. These samples were stored in an incubator at 20ºC for 20 days and analysed for the changes in sensory attributes, texture and microbiological changes.

Statistical analysis

The data obtained from the various experiments were recorded as mean ± standard error (SE). Data obtained were statistically analysed using complete randomized design (CRD) and analysis of variance (ANOVA), significance of treatment effects was determined at 5% level (Panse and Sukhatme, 1985) [22].

Results and discussions
Effect of banana flour on the sensory scores of bread

Table 1 shows the sensory scores of bread prepared from (WF: BF) flours. From the results it was found that at 40% BF supplementation level (T_4), all the sensory parameters were highest as compare to other combinations. Overall colour score of control was 8.52 ± 0.20. As the supplementation level of BF increased above 40%, overall colour scores significantly (P< 0.05) decreased from 5.51 ± 0.23 to 4.57 ± 0.22 with respect to control. Flavour score for control was 8.50 ± 0.11. There was a significant gradual increase in the flavour score up to 8.80 ± 0.32 with 40% supplementation level (T_4). The increase in flavour indicates a better flavour rating, thereafter the flavour scores decreased from 6.12 ± 0.22 to 4.42 ± 0.15 in the remaining samples. The decrease in flavour score may be due to the characteristic flavour of BF flour. Dhingra and Jood (2001) [11] reported that the flavour score of bread increased on increasing the level of barley and soy flour up-to 10% level and then it decreased at 15% and 20% of supplementation due to the beany flavour of soyabean flour. The decrease in mouthfeel score may be attributed to gradual loss in chewiness with an increase in supplementation level of BF. According to the overall acceptability score, inclusion of 40% BF in bread could be accepted and further increase in BF level would not give better consumer acceptance. Hence the combination containing 60% WF and 40% BF was selected as per the recommendation by the sensory panellists.

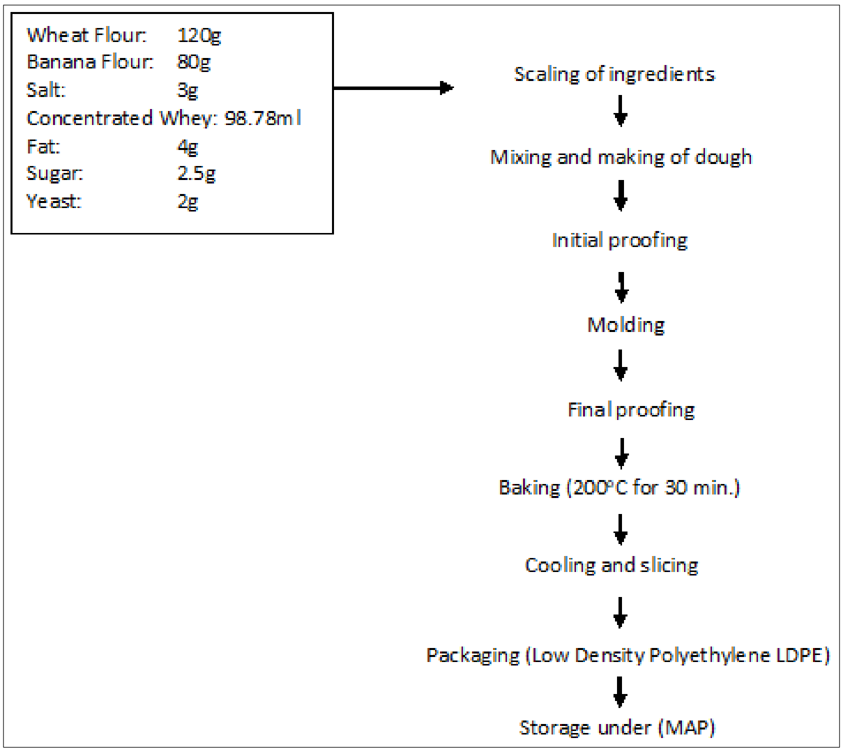

Fig 1: Flow chart for preparation of bread with concentrated whey incorporation

Table 1: Effect of supplementation of banana flour on sensory scores of bread

Sample code	BF (%)	WF (%)	Overall Color	Crumb Texture	Flavour	Mouthfeel	Overall Acceptability
T_0 (Control)	0	100	8.52±0.20^a	9.01±0.17^a	8.50±0.11^b	8.56±0.22^a	8.66±0.28^b
T_1	10	90	8.41±0.23^a	8.00±0.00^b	8.62±0.22^{ab}	8.42±0.13^{ab}	8.52±0.15^b
T_2	20	80	8.40±0.08^a	7.60±0.08^c	8.55±0.33^b	7.70±0.26^c	8.15±0.76^c
T_3	30	70	8.21 ±0.28^b	7.50±0.458b^c	8.50±0.45^b	7.70±0.23^c	7.40±0.20^c
T_4	40	60	8.30±0.05^b	7.63±0.05^c	8.80±0.32^a	7.80±0.15^d	8.80±0.10^a
T_5	50	50	5.51±0.23^c	5.00±0.00^d	6.12±0.22^c	6.30±0.13^e	6.00±0.15^d
T_6	60	40	4.57±0.22^d	4.70± 0.08^d	4.42±0.15^d	4.80±0.25^f	5.12±0.15^e

BF: banana flour: WF: wheat flour. Values are mean ± SD of (n=3) and means in the same column with different superscripts are significantly different at (P<0.05)

The proximate composition of bread without and with addition of BF and concentrated whey (25% TS) are presented in Table 2. Moisture content of BF bread was higher as compared to control bread. High moisture content might be related to high protein of concentrated whey and starch composition. Moisture content of bread samples could be increased due to replacement of BF. Similar results were reported by Adebourale *et al.*, (2009) [1] in sweet potato wheat bread. In this study, bread from 40% BF had higher crude fiber than control. This implies that BF could serve as a useful supplement in food material where fiber is of great importance. Ash content was higher in BF supplemented bread than control bread. This was due to higher ash content of banana flour, especially the potassium and magnesium contents. Similar results were reported by Alkarkhi *et al.*, (2011) [2]. Protein content of BF bread was higher due to incorporation of concentrated whey, which is very important for growth and maintenance of the body. The bread prepared with added BF had higher level of resistant starch (6.81 ± 0.14%) than the control bread (1.20 ± 0.07%). Wheat bread contains high amount of digestible starch fractions, but BF is an alternative for product development with reduced digestible starch content. The bread formulated with BF had higher dietary fiber (5.20 ± 0.12%) content than control sample (2.20 ± 0.12%). Sangronis and Rebolledo (1993) [24] reported that the commercial bread samples have dietary fiber contents ranges from 4.99% to 6.11% and in fibre rich cookies, it was 3.37% to 5.95%.

Table 2: Proximate composition of optimized BF supplemented (T_4) bread

Parameters (%)	Control	T_4
Moisture	13.50± 0.10	26.26±0.27
Fat	1.30± 0.01	1.29±0.00
Ash	2.00± 0.01	3.33±0.13
Total protein	4.20 ±0.10	9.70±0.10
Total carbohydrate	58.90 ±0.80	63.21±0.26
Dietary Fiber	2.20± 0.12	5.20±0.12
Resistance starch	1.20±0.07	6.81±0.14

Control: bread without banana flour; T4: bread containing 40 % banana flour. Values are mean± SD of (n=3) and means in the same column with different superscripts are significantly different at (P<0.05)

Effect of level of BF on total phenolic content and free radical scavenging activity of bread

From Table 3 it could be noted that the total phenolic content in control sample was found to be 1.40 ± 0.10 (mg/GAE/100g). Total phenolic content in BF supplemented bread increased from 23.33 ± 0.57 to 25.00 ± 1.52 (mg/GAE/100g), upon increasing levels of BF from 10 to 40%. The total phenolic content obtained from control suggested that the wheat flour also contain TPC in small quantity. The presence of polyphenolic compounds in BF had in turn added up to the increasing level of polyphenols to enhance the antioxidant content of bread. The antioxidant activity increased with the increase in BF concentration in bread in trend which was similar to the study of Kurhade *et al.*, (2016). Phenolics are important plant constituents and able to scavenge free radicals because of the presence of hydroxyl group. As the concentration of BF increased from 10% to 60%, the total phenolic content of bread was also increased from 23.33 ± 0.57 to 25.33 ± 1.52 (mg/GAE/100g). Similar results had been reported by Sharma and Gujral (2014) [25]. In a similar manner the antioxidant activity was also increased with an increased amount of BF. The scavenging activity of bread (bread incorporated with 40% BF) reached a value of 53.43 ± 1.25%. The highest % DPPH was recorded with the use of 60% BF (55.92 ± 0.15).

Table 3: Effect addition of banana flour (BF) on TPC & DPPH of bread

Sample code	BF (%)	TPC (mg/GAE/100g)	DPPH (%)
T_0 (Control)	0	1.40±0.10^{d}	4.70±0.20^{g}
T_1	10	23.33±0.57^{c}	46.90±3.04^{f}
T_2	20	24.33±0.57^{b}	47.03±4.31^{e}
T_3	30	24.33±0.57^{b}	51.59±1.34^{d}
T_4	40	25.00±1.00^{a}	53.43±1.25^{c}
T_5	50	25.00±1.73^{a}	54.32±0.45^{b}
T_6	60	25.33±1.52^{a}	55.92±0.15^{a}

BF: banana flour; TPC: total phenolic content; DPPH: (2,- 2 -Diphenyl-1-picryl hydrazyl). Values are mean ± SD of (n=3) and means in the same column with different superscripts are significantly different at (P<0.05)

Effect of storage period on the texture profile of bread

During storage of bread under modified atmosphere packaging (MAP), a gradual increase in the hardness in samples stored under air as package atmosphere was observed (Table 4) due to reduction in the moisture content of the bread. Similar results were reported by Weegels *et al.*, (1996) [27]. The maximum cohesiveness of the samples packed with 98% N_2 and 50% N_2: 50 % CO_2 was 0.74 ± 0.86 and 0.70 ± 0.54, respectively. However, the cohesiveness of the MAP packed bread remained fairly constant throughout the storage. Londhe *et al.*, (2012) [18] have reported that cohesiveness of MAP packed product remained constant up to 20–30 days and the current findings are in accordance with this observation. Loss in moisture content may be responsible for the decrease in the cohesiveness with the progression of storage. The gumminess of the samples followed the same trend as that of cohesiveness. There was a gradual decline in gumminess of bread during the storage.

Table 4: Effect of storage period on the texture profile of bread

Storage Period (Days)	Composition of gasses	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience	Hardness(N)
0	Control	0.95±0.02	0.70±0.02	4068±18.40	3867±21.72	0.36±0.01	5792±0.02
5	98%N_2	0.96±0.12	0.74±0.11	3677±12.02	3531±34.21	0.35±0.02	5865±0.02
	50%N_2: 50% CO_2	0.95±0.00	0.70±0.05	4068±25.06	3867±23.22	0.36±0.12	5792±0.02
10	98%N_2	0.96±0.11	0.74±0.07	3677±11.05	3531±32.62	0.35±0.04	5863±0.02
	50%N_2: 50% CO_2	0.94±0.21	0.60±0.11	3671±21.05	3273±42.52	0.24±0.03	5785±0.02
15	98%N_2	0.90±0.22	0.64±0.32	2823±17.04	3838±21.66	0.26±0.06	7639±0.02
	50%N_2: 50% CO_2	0.94±0.42	0.60±0.41	3471±30.23	3273±41.22	0.24±0.07	5785±0.02
20	98%N_2	0.96±0.51	0.60±0.23	2393±21.12	2166±13.62	0.30±0.02	7933±0.02
	50%N_2: 50% CO_2	0.97±0.22	0.54±0.34	3210±21.11	2532±16.50	0.21±0.01	5880±0.02

Values are mean ± SD of (n=3) and means in the same column with different superscripts are significantly different at (P<0.05)

Effect of storage period on TPC and DPPH of bread during storage

The values of % DPPH measured to 515 nm varied from 53.43 ± 1.25 to 51.62 ± 2.81 during the 20 days storage period, in case of samples stored under 98% N_2 atmosphere. The samples stored in both the modified atmospheric packages displayed slow deteriorative changes with %DPPH values ranging from 53.24 ± 1.14 to 51.62 ± 2.81and 53.38 ± 0.84 to 53.31 ± 1.33 for samples with 98% N_2 and 50% N_2:50%CO_2 gas composition, respectively (Figure 2 & 3). The TPC content was not showing any significant difference (P<0.05) in the result during the storage period of 20 days. Total phenolic compounds concentration remains relatively stable during storage, but some individual compounds may vary (Jager *et al.*, 2000). Moreover, processing conditions and prolonged storage promote both chemical and enzymatic oxidation of phenolic compounds, contributing to its reduction (Lee *et al.*, 2003) [17].

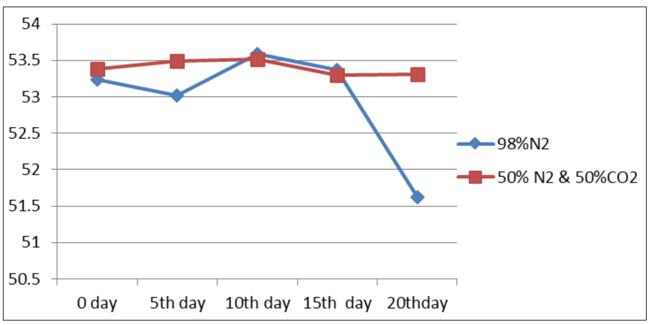

Fig 2: Effect of storage period on DPPH of bread at different gases composition during storage at 20°C

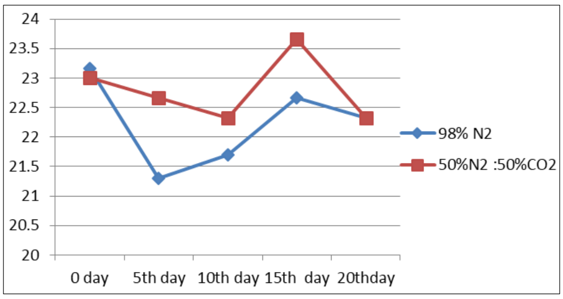

Fig 3: Effect of storage period TPC of bread at different gases composition during storage at 20°C

Microbiological changes in bread during storage

The samples containing 98% N_2 displayed delayed microbial growth as nitrogen is an inert gas which suppresses the microbial growth (Table 5). The maximum value for total microbial load on Nutrient Agar Media was 3.63 ± 0.57 log_{10} CFU/ml on 20th day of storage, while maximum value for fungal count was 2.76 ± 1.58 log_{10} CFU/ml on 20th day of storage. However, the samples with the gas combination of 50% N_2: 50% CO_2 displayed increased inhibition of microbial growth when compared to bread samples containing 98% N_2, probably due to the bacteriostatic effect of CO_2 as it is also mentioned in studies of Daniels *et al.*, (1985) [10]. Smith *et al.*, (1986) [26] also reported that the crusty rolls packed in 40% N_2: 60% CO_2 with the headspace O_2 concentration remained mould-free even after 60 days. Similarly, Black *et al.*, (1993) [26] reported lower mould and yeast counts in pita bread packed using different CO_2 concentrations. They reported that the mould counts were 2.1× 10^{-3}cfu/g after 2 days in 15% CO_2, 2× 10^{-3} cfu/g after 14 days in 40% CO_2, and <10 cfu/g after 14 days in 100% CO_2. Yeast counts remained unchanged (<10 cfu/g) in the different atmospheric conditions. The minimum concentration required for the growth of *Aspergillus niger* and *Penicillium spp*. in CO_2: N_2 (3:2) was 0.4% (Smith *et al.*, 1986) [26]. Coliform count was found nil during storage period in BF supplemented bread.

Table 5: Microbial changes in BF supplemented bread during storage at 20°C

Storage period	Composition of gasses	Fungal count (log_{10}CFU/ml)	Coliform count (log_{10}CFU/ml)	Total plate count (log_{10}CFU/ml)
0 day		Nil	Nil	Nil
5th day	98%N_2	1.82±5.77 d	Nil	2.52±0.57 e
	50%N_2: 50% CO_2	Nil	Nil	Nil
10th day	98%N_2	2.32±0.57 c	Nil	3.12±1.58 c
	50%N_2: 50% CO_2	Nil	Nil	2.12±1.00 f
15th day	98%N_2	2.50±1.00 b	Nil	3.36±0.57 b
	50%N_2: 50% CO_2	1.52±0.55 cd	Nil	2.70±1.00 e
20thday	98%N_2	2.76±1.58 a	Nil	3.63±0.57 a
	50%N_2: 50% CO_2	2.07±1.73c	Nil	3.02±1.00 d

Values are mean ± SD of (n=3) and means in the same column with different superscripts are significantly different at (P<0.05)

Conclusion

From the present study, it can be concluded that 40% BF and 98.78 mL concentrated whey added bread sample score highest in most of the attributes. The textural characteristics of bread samples indicated that control, 10% and 20% BF supplemented bread did not show significant difference. The combination 50% N_2: 50% Co_2 creates the reliable atmosphere for extending shelf-life of bread. The total plate count and fungal count of BF bread samples were found within the acceptable limits. This study concluded that supplementation level of 40% BF results in acceptable textural and nutritional characteristics. BF bread presented significantly higher resistant starch, dietary fiber, protein and ash content from control bread. Unripe banana flour appears to a good source for obtaining BF with high resistant starch.

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