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Effects of different packaging materials on the sensory characteristics of β-carotene enriched pearl millet based cookies during storage

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Abstract
In present investigation, an attempt was made to utilize microencapsulated β-carotene and pearl millet flour in formulation of nutrient rich cookies. The storage stability of cookies as influenced by different packaging materials, *viz.* low density polyethylene (LDPE-25μ), high density polyethylene (HDPE-25μ) and aluminium laminated pouches (20μ) was evaluated at ambient conditions. Cookies were evaluated for their sensory characteristics and β-carotene retention during storage. There was decrease in sensory quality characteristics during storage irrespective of package at varying range. Also the β-carotene retention was affected by packaging material. A gradual decrease in β-carotene of cookies from 2.56 to 2.17 mg/100g was observed for 180 days of storage. Aluminium laminated and HDPE pouches were found well suitable packaging materials. A serving of these cookies provided 0.68 mg (85 RE) of the β-carotene.

Keywords microencapsulated β-carotene; pearl millet; cookies, storage studies; β-carotene retention

Introduction
Vitamin A deficiency (VAD) is one of the major nutritional deficiencies affecting the population in developing countries. Prevalence of VAD in India is one of the highest in the world. In India, 62 % of pre-school children are vitamin A deficient, having serum retinol concentrations lower that 20 μg/dL (Singh, 2014) [24]. B-carotene is the principal precursor of vitamin A which theoretically possesses 50 % Vitamin A activity (Delgado Vargas *et al*., 2000) [6]. The high degree of unsaturation in β-carotene structure renders it extremely susceptible to oxygen (Maa *et al.,* 1998) [16]. Microencapsulation, being one of the best ways for retaining the stability of β-carotene.
With the increasing knowledge of the positive functions of β-carotene, more and more people take interest in some foods or pharmaceuticals containing β-carotene ingredients, such as beverages, baked goods, oils, capsules and tablets. Lipid-soluble vitamins such as vitamin A, β-carotene and vitamins D, E or K are much easier to encapsulate than water-soluble ingredients (Kowalski *et al*., 2000) [13].
Also the higher utilization and nutritional significance of cookies is well recognized for enrichment with high quality non-wheat flours. Nevertheless, the utilization of millets is limited due to the presence of various anti-nutrients. Pearl millet (*Pennisetum glaucum* L) is recognized as being the most widely grown of all the millet types. It is the basic staple food in the poorest countries and used by the poorest people. Nutritionally, it makes an important contribution to human diet due to high levels of calcium, iron, zinc, lipids and high quality proteins. It contains vitamin A typically about 24 Retinol Equivalents (Taylor, 2004). The beneficial attribute of pearl millet flour (PMF) due to its complementary mineral pattern to that of wheat minerals has led to worldwide attempts to enrich bakery products using PMF.
Hence, the present investigation was carried out to standardize the storage stability of microencapsulated β-carotene incorporated pearl millet based cookies as affected by different packaging materials. Also the study emphasizes the contribution of β-carotene from these cookies.

Materials and Methods
Materials: The pearl millet *var*., *Dhanshakti* (ICTP 8203 Fe 10-2) was procured from Department of Plant Breeding, College of Agriculture, Dhule, Mahatma Phule Krishi Vidyapeeth, Rahuri.

The microencapsulated β-carotene powder (MBC) was prepared in the laboratory using spray drier. The raw materials such as *maida*, sugar, *vanaspati*, sodium bicarbonate, ammonium bicarbonate, etc. were purchased from local market of Rahuri.

Packaging materials: For the study, three types of packaging materials were used, *viz.* low density polyethylene (LDPE), high density polyethylene (HDPE) and aluminium laminate pouches were procured from the market of Pune. The specifications of the packaging materials are given in Table 1.

Table 1: Specifications of Packaging Materials

Packaging material	Thickness (µ)	WVTR[a]	GTR[b]
LDPE	25	18	8000
HDPE	25	9	3000
Aluminium laminate	20	0	0

a- Water vapour transmission rate (g/m^2/24 hours) at 38ºC, 90% RH
b- Gas transmission rate (cc/m^2/24 hours) (100% oxygen) at 25ºC, 45% RH

Preparation of pearl millet flour (PMF)

The pearl millet grains were cleaned to remove any impurities by hand picking. Then the grains were milled in flour mill to make flour and the flour obtained was sieved through 80 mesh size sieve to obtain fine PMF.

Preparation of MBC: Pure *Tran's* β-carotene was added to the solution of maltodextrin (30% w/w) in distilled water. The mixture was then homogenized to obtain an aqueous emulsion (feed liquid) and immediately fed to the spray-dryer (M/s. Labultima, LU-228) to obtain MBC. The inlet and outlet air temperature were maintained at 170±5ºC and 95±5ºC, respectively.

Preparation of cookies

Cookies were prepared using the traditional creamery method described by Whitley (1970) [29]. The ingredients included 56 g of wheat *Maida*, 40 g of pearl millet flour, 4 g of microencapsulated β-carotene powder, 50 g of sugar, 50 g of *vanaspati*, 0.5 g of ammonium bicarbonate, 0.5 g of sodium bicarbonate, and required amount of water. Flour was sieved with sodium bicarbonate and ammonium bicarbonate. The cream was mixed with flour and sufficient quantity of water was added to form dough. Then dough was divided into small pieces. The pieces were rounded, flattened and placed in the baking tray smeared with fat and baked at 180-200°C for 15 min. The cookies were allowed to cool, packed in various packages and stored at ambient temperature (Kure *et al.*, 1998) [15]. The cookies were evaluated for nutritional and sensory quality.

Storage studies

The cookies were packed in different packages *viz.* P_1- low density polyethylene (LDPE), P_2- high density polyethylene (HDPE) and P_3- aluminium laminated packages. The packages were stored at ambient temperature. The sensory quality and β-carotene retention in cookies were evaluated at an interval of 30 days for a period of 6 months.

Sensory evaluation: The cookies were evaluated for sensory attributes by a panel of 14 semi-trained judges (7 men and 7 women) selected from the post graduate students and the staff members of the department of Food Science and Technology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist.- Ahmednagar. Separate score cards were provided to the judges for sensory evaluation of cookies using a 9 point Hedonic scale system (Amerine *et al.,* 1965) [2]. The evaluation was carried out for different parameters like colour and appearance, texture and grain, flavour, crispiness, taste and overall acceptability. The appropriate analysis was carried out to determine the significance of variations of average score and the contribution of individual parameter. Samples were served to the panelists and they were asked to rate the acceptability of the product on 1–9 points scale, ranging from the extreme like (9) to dislike extremely (1) as described by Adeyeye *et al*., (2017) [1].

B-carotene content

The β-carotene content of cookies was determined by method as suggested by Srivastava and Kumar (2009) [26]. 5 g of sample of sample was grinded with few crystals of anhydrous sodium sulphate and mixed with 10-15 ml acetone. The supernatent was decanted in a beaker. The process was repeated twice and the combined supernatant was transferred to a separating funnel. 5-10 ml of petroleum ether was added and mixed thoroughly. Two layers were separated out on standing. The lower layer was discarded and the upper layer collected in 100 ml volumetric flask, volume was made up to 100 ml with petroleum ether and optical density was recorded at 452 nm. Petroleum ether was used as blank.

Statistical analysis

The data obtained was analyzed statistically using ANOVA: Two-factor with replication to determine statistical significance of treatments. Completely randomized design was used as given by Snedecor and Cochran (1987). The data was then compared using Duncan's multiple range tests at 5 % significance level (Duncan, 1995) [7].

Results and discussion

Sensory quality of cookies during storage

The results of mean scores for different sensory attributes such as colour and appearance, texture and grain, flavour, crispiness, taste and overall acceptability of cookies as influenced by packaging material and storage period are presented in Table 2 and discussed as referred below.

Table 2: Sensory Properties of Cookies Influenced By Package and Storage Period

Storage period (Days)	Colour and Appearance (8.18)*			Texture and Grain (8.00)*			Flavour (7.85)*			Crispiness (8.60)*			Taste (8.40)*			Overall Acceptability (8.21)*		
	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3
30	7.50 (±0.14) b	8.00 (±0.18) a	8.10 (±0.16) a	7.50 (±0.20) b	7.60 (±0.16) b	8.00 (±0.18) a	7.00 (±0.10) c	7.25 (±0.12) b	7.67 (±0.14) a	7.60 (±0.10) c	7.80 (±0.10) b	8.20 (±0.12) a	7.40 (±0.14) c	7.80 (±0.10) b	8.25 (±0.08) a	7.40 (±0.10) c	7.79 (±0.14) b	7.94 (±0.08) a
60	7.30 (±0.20) b	7.80 (±0.12) a	7.97 (±0.16) a	7.00 (±0.17) c	7.40 (±0.20) b	7.80 (±0.22) a	6.85 (±0.08) c	7.10 (±0.06) b	7.50 (±0.07) a	7.20 (±0.18) c	7.60 (±0.18) b	8.00 (±0.20) a	7.00 (±0.12) c	7.50 (±0.12) b	8.00 (±0.14) a	7.07 (±0.16) c	7.59 (±0.15) b	7.70 (±0.18) a

90	7.00 (±0.12)^{c}	7.50 (±0.10)^{b}	7.75 (±0.13)^{a}	6.67 (±0.20)^{c}	7.20 (±0.24)^{b}	7.60 (±0.20)^{a}	6.67 (±0.18)^{c}	6.97 (±0.23)^{b}	7.35 (±0.20)^{a}	6.60 (±0.18)^{c}	7.33 (±0.20)^{b}	7.80 (±0.16)^{a}	6.50 (±0.16)^{c}	7.25 (±0.15)^{b}	7.60 (±0.12)^{a}	6.69 (±0.20)^{c}	7.31 (±0.18)^{b}	7.49 (±0.18)^{a}
120	6.60 (±0.18)^{c}	7.10 (±0.18)^{b}	7.50 (±0.20)^{a}	6.25 (±0.17)^{c}	7.00 (±0.15)^{b}	7.40 (±0.15)^{a}	6.25 (±0.24)^{c}	6.75 (±0.32)^{b}	7.20 (±0.37)^{a}	6.40 (±0.15)^{c}	7.17 (±0.12)^{b}	7.60 (±0.20)^{a}	6.20 (±0.20)^{c}	7.00 (±0.23)^{b}	7.40 (±0.27)^{a}	6.34 (±0.21)^{c}	7.01 (±0.20)^{b}	7.25 (±0.16)^{a}
150	6.20 (±0.20)^{c}	6.97 (±0.14)^{b}	7.18 (±0.18)^{a}	6.00 (±0.18)^{c}	6.80 (±0.14)^{b}	7.20 (±0.16)^{a}	6.00 (±0.22)^{c}	6.67 (±0.25)^{b}	7.00 (±0.20)^{a}	6.00 (±0.18)^{b}	7.00 (±0.12)^{a}	7.15 (±0.14)^{a}	6.00 (±0.32)^{c}	6.85 (±0.24)^{b}	7.20 (±0.28)^{a}	6.04 (±0.27)^{c}	6.97 (±0.25)^{a}	7.04 (±0.20)^{a}
180	6.13 (±0.23)^{b}	6.70 (±0.12)^{a}	6.85 (±0.10)^{a}	5.62 (±0.20)^{c}	6.60 (±0.17)^{b}	6.97 (±0.14)^{a}	5.80 (±0.24)^{c}	6.40 (±0.20)^{b}	6.90 (±0.18)^{a}	5.63 (±0.20)^{b}	6.60 (±0.18)^{a}	6.75 (±0.16)^{a}	5.83 (±0.27)^{c}	6.76 (±0.33)^{b}	7.00 (±0.32)^{a}	5.80 (±0.20)^{b}	6.74 (±0.18)^{a}	6.76 (±0.20)^{a}
Mean	6.99 (±0.17)^{c}	7.46 (±0.18)^{b}	7.65 (±0.18)^{a}	6.72 (±0.16)^{c}	7.23 (±0.14)^{b}	7.57 (±0.18)^{a}	6.63 (±0.20)^{c}	7.00 (±0.18)^{b}	7.35 (±0.18)^{a}	6.86 (±0.18)^{c}	7.44 (±0.16)^{b}	7.73 (±0.20)^{a}	6.76 (±0.22)^{c}	7.37 (±0.20)^{b}	7.69 (±0.24)^{a}	6.79 (±0.18)^{b}	7.37 (±0.16)^{a}	7.48 (±0.14)^{a}

Each value is the average of ten observations P_1- LDPE package, P_2- HDPE package, P_3- Laminate package*Sensory scores of cookies at 0 day
Means not sharing a common superscript letter(s) in a row are significantly different at $p<0.05$ as assessed by Duncan's multiple-range test

Colour and Appearance

A gradual decrease in mean score for colour and appearance from 8.18 to 6.56 was observed for 180 days of storage. The maximum score (6.85) was observed in cookies packed in P_3, followed by P_2 (6.70) and P_1 (6.13) packages on the 180th day of storage. The results obtained in the present investigation are concurrent with literature. The colour changes during storage might be due to acceleration of Maillard reaction, absorption of moisture contents during storage stimulates Maillard reaction in cookies (Bender, 1996) [4]. Jood *et al.* (2001) [11] also reported gradual decrease in the organoleptic characteristics of β-carotene and iron rich biscuits and *shankarpara*. In another study (Elahi, 2006) [8], gradual decrease in colour of biscuits as a function of storage was observed which supports the finding of present study. Butt *et al.* (2007) [5] reported similar variation in colour of vitamin A fortified cookies during storage. Kulthe *et al.* (2014) [14] reported similar decrease in colour and appearance score while studying the effect of packaging material on sensory quality of cookies during storage for 90 days.

Texture and grain

The mean texture score was found to be decreased from 8.00 to 6.40 during the storage of cookies for 6 months. The maximum score for texture and grain (6.97) was observed in treatment P_2 followed by treatments P_3 (6.60) and P_1 (5.62). The gradual decrease in texture score was due to softening effect resulting from gain of moisture during storage was observed. Cookies packed in LDPE (P_1) showed more moisture gain during storage and became soft compared to HDPE (P_2) and Laminate (P_3). This might be due to high water vapour transmission rate (WVTR) of LDPE as compared to HDPE and laminated packages. Paine (1969) [20] had also mentioned that aluminium foil had low water vapour transmission rate (WVTR) and gas transmission rate (GTR) as compared to polyethylene. Sathe *et al.* (1981) [23] reported softening of crackers packed in PP bags and stored at ambient conditions. Similar results are presented by Wade (1988) [28] for biscuits, cookies and crackers; Bender (1996) [4]. For cookies and Butt *et al.* (2007) [5]. They reported the same decreasing trend in texture with increase in storage time. Nagi *et al.* (2012) [17] reported gain in moisture of biscuits during storage in HDPE and laminate package might be due to hygroscopic nature of dried product, storage environment (temperature, relative humidity) as well as nature of packaging material. Kulthe *et al.* (2014) [14] reported similar results during storage (90 days) of cookies as influenced by different packaging materials.

Flavour

It is clear from the data that a gradual decrease in score for flavour from 7.85 to 6.37 was observed. The flavour retention was higher in case of laminated package (P_3) as compared to LDPE (P_1) and HDPE (P_2) packages. In all the treatments, P_3 produced highest score of 6.90 followed by P_2 (6.40) and P_1 (5.80) treatments. The flavouring compounds might be lost at higher rate during storage that lowered the flavour score. This might be due to high GTR of LDPE compared to other packaging materials (Paine, 1969) [20]. Sathe *et al.* (1981) [23]. Reported development of rancid flavour in crackers prepared from soy flour and groundnut flour after 60 days of storage. The flavour changes during storage might be due to absorption of moisture contents during storage that caused flavour deterioration in cookies (Wade, 1988) [28]. Gupta and Singh (2005) [10] reported reduced flavour score of maize fortified biscuits during storage. Kaur (2005) [10] reported that flavour changes were higher in case of cookies stored in LDPE as compared to those stored in laminate package because of the fact that aluminiun laminates protects the biscuits from light which acts as catalyst for oxidation.

Crispiness

The data indicates that the mean value for crispiness score of cookies decreased from 8.60 to 6.33 during the storage period of 180 days. The decrease in crispiness score may be due to moisture gain during storage. The highest score of crispiness (6.75) was obtained in case of treatment P_3. This indicates that laminated package had higher moisture barrier properties than LDPE and HDPE packages (Paine, 1969) [20]. Sathe *et al.* (1981) reported reduced crispiness in crackers after 45 days of storage. Wade (1988) [28] and Bender (1996) [4]. observed similar decreasing trend for crispiness during storage of biscuits. Gupta and Singh (2005) [10] also reported lowered crispiness in biscuits prepared from quality protein maize when packed in polyethylene bags and stored at ambient conditions for 60 days. Kaur (2005) [12] reported that reduction in crispiness was due to higher moisture absorption of cookies stored in LDPE as compared to those stored in laminate package. Nagi *et al.* (2012) [17] reported gain in moisture of biscuits during storage in HDPE and laminate package might be due to hygroscopic nature of dried product, storage environment (temperature, relative humidity) as well as nature of packaging material. Kulthe *et al.* (2014) [14] reported higher reduction in crispiness of cookies packed in LDPE and PP pouches than those packed in HDPE pouches during storage for 90 days.

Taste

A gradual decrease in score for taste of cookies from 8.40 to 6.53 was observed during the storage period. The highest score of taste (7.00) was observed for cookies packed in laminated package indicating slight deteriorative change. This might be due to low WVTR and GTR of laminated package as compared to LDPE and HDPE packages. It was reported that taste score decreased during storage with respect to storage conditions and period of storage. The results obtained in present investigation for cookies are in agreement with literature. Wade (1988) [28] and Bender (1996) [4] reported the same decreasing trend in taste of cookies, biscuits and crackers with increase in storage time. Jood *et al*. (2001) [11] also reported gradual decrease in the taste score of β-carotene and iron rich biscuits and *shankarpara*. Narender *et al.* (2007) [18] also reported change in taste of biscuits stored for 60 days under ambient conditions. Kulthe *et al*. (2014) [14] reported similar trend for taste variation while studying effect of packaging material on sensory quality of cookies during storage for 90 days.

Overall acceptability

The data showed decrease in overall acceptability score from 8.21 to 6.44 during storage of cookies for 6 months. The maximum score of 6.76 was observed for treatment P_3 i.e. laminated package stored for 6 months at ambient temperature. The treatment P_2 i.e. HDPE package had also overall acceptability score of 6.74 which was comparable with P_3 (Laminated package) treatment. While treatment P_1 (LDPE) showed fair score (5.80) for overall acceptability in comparison to other two. It might be due to faster deterioration in respect of colour and appearance, texture and grain, flavour, crispiness and taste of cookies packed in LDPE package. It was observed that the overall acceptability score for all samples decreased during storage with respect to storage condition and period. It is in conformity with the results obtained by various researchers.

The results of the present investigation are in conformity with the results obtained by various researchers. Sathe *et al.* (1981) [23] reported that crackers packed in PP bags could be stored for about 60 days at normal conditions. Similar results are presented by Wade (1988) [28], Bender (1996) [4], Jood *et al.* (2001) [11], Butt *et al.* (2007) [5] and Gupta and Singh (2005) [10]. They reported Narender *et al.* (2007) [18] also concluded that biscuits could be stored at ambient temperatures (30-35°C) for 60 days without any undesirable changes in the sensory attributes. Kulthe *et al*. (2014) [14] reported that cookies could be stored for more than 90 days without affecting their sensory quality.

Retention of β-carotene in cookies during storage

A gradual decrease in β-carotene content of cookies was observed during 180 days of storage (Table 3). At 0 day, the β-carotene content of cookies was 2.56 mg/100g. On storage for 180 days, the retention of β-carotene varied with type of package. The maximum β-carotene retention (2.33 mg/100g) was observed in cookies packed in P_3, followed by P_2 (2.26 mg/100g) and P_1 (1.92 mg/100g) packages on the 180th day of storage. The results showed highest β-carotene retention in P_3 (91 %) samples followed by P_2 (88 %) and P_1 (75 %) during storage of 6 months. The results obtained in present investigation are concurrent with literature.

Gordon *et al*. (1985) reported that β-carotene was stable in yellow cakes, sugar cookies and yeast raised sweet dough when stability was based only on colour retention. Rogers *et al*. (1993) [22] reported no significant losses occurred during the typical shelf life of yellow cake, sugar cookies and bagel. They reported 68-75 % of β-carotene retention in baked products during storage. One possible explanation for the variation in β-carotene retention in cookies of the two studies is the longer storage time used in this study compared with four week storage in their study. In fact, the differences in β-carotene degradation in cookies were rather small at four weeks storage in the Rogers *et al*. (1993) [22] study; most of the differences in β-carotene degradation occurred in the last eight weeks of storage.

Ranhotra *et al*. (1995) reported 68-90 % of β-carotene retention in bread and crackers during storage with addition of anti-oxidants. Jood *et al*. (2001) [11] reported reduction in β-carotene of β-carotene and iron rich biscuits from 1.42 to 1.14 mg/100g after 60 days of storage. Butt *et al*. (2007) [5] reported maximum losses of 10.85 % occurred after 30 days storage of vitamin A fortified cookies. In another study (Bauernfeind, 2006) [3], 90-100 % retention of vitamin A was observed during baking with a minimal loss of vitamin A after 6 months storage. In most cases, the stability of carotene changed only minimally after baking during storage. The results are more or less consistent with the literature.

Table 3: β-carotene retention (mg/100g) in cookies influenced by package during storage

Storage period (Days)	β-carotene (2.56)*		
	P_1	P_2	P_3
30	2.45 (±0.00)^b	2.49 (±0.01)^{ab}	2.53 (±0.00)^a
60	2.38 (±0.01)^b	2.46 (±0.01)^a	2.49 (±0.01)^a
90	2.30 (±0.01)^b	2.42 (±0.00)^a	2.45 (±0.02)^a
120	2.23 (±0.03)^b	2.37 (±0.02)^a	2.40 (±0.02)^a
150	2.05 (±0.02)^b	2.32 (±0.02)^a	2.37 (±0.02)^a
180	1.92 (±0.01)^b	2.26 (±0.00)^a	2.33 (±0.01)^a
Mean	2.27 (±0.02)^b	2.41 (±0.03)^a	2.45 (±0.02)^a

Each value is the average of ten observations

P_1- LDPE package, P_2- HDPE package, P_3- Laminate package

* Β-carotene content of cookies at 0 day

Means not sharing a common superscript letter(s) in a row are significantly different at $p<0.05$ as assessed by Duncan's multiple-range test.

Contribution of β-carotene from cookies

The contribution of β-carotene on the basis of their serving size in the form of cookies is presented in Table 4. The β-carotene was stable and no significant loss was occurred in β-carotene after baking, all values obtained after baking were averaged to arrive at typical β-carotene. On dry basis the β-carotene content in cookies was 2.38 mg/100g. On an as-consumed basis, a serving of these in cookies would provide 0.68 mg of the β-carotene i.e. 85 Retinol Equivalent (RE). Thus the minimum daily dose of 50 RE as recommended by NIN, 2009 [19], can be fulfilled by a serving of these cookies. Also higher additions of carotene would obviously provide more carotene. However, it may be prudent to not over fortify foods, as combined intakes of carotene from various fortified foods and supplements may become excessive, going well beyond levels suggested for the antioxidant effect.

Rogers *et al*. (1993) [22] reported that each serving of β-carotene enriched cookies provided 3.65 mg/100g β-carotene on dry basis and about 1 mg of β-carotene on an as-consumer basis. As mentioned earlier, the longer storage time used in this study compared with four week storage in their study resulted in lowering the average of all values after baking and storage. Hence there was variation in the contribution value obtained in this study and that reported by Rogers *et al.* (1993) [22].

Table 4: contribution of β-carotene in cookies tested

Product	β-carotene[a] (mg/100g)	Serving size		β-carotene[b] (mg)
		(g)	Household unit	
Cookies	2.38	28.5 (1oz)	3 pieces	0.68 (85 RE)[c]

Conclusion

Thus from the results obtained in the present investigation and with reference to observations of various researchers, it is reported that cookies prepared by substitution of *maida* with PMF and supplemented with MBC, could be stored for more than 180 days at ambient conditions, aluminium laminated and HDPE packages being well suitable packaging materials. On dry basis the β-carotene content in cookies was 2.38 mg/100g. On an as-consumed basis, a serving (1 oz or 3 pieces) of these cookies would provide 0.68 mg (85 RE) of the β-carotene.

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