



Peroxide formation and vitamin A stability in fortified refined sunflower oil along the commercial chain of large and small/medium factories in Kazakhstan

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Abstract

The instability of vitamin A in edible oil has been attributed to exposure to oxygen and light, and the formation of peroxides accelerated by the same causes, and the catalytic effect of high temperature. The peroxide value and retinol retention in fortified oil at different points of the market supply chain (from factory to retailer), and during household use in Kazakhstan were evaluated. Peroxide formation and vitamin A retention were measured in oil samples stored in dark boxes and under daylight, simulating large factory (LF) and small-to-medium factory (SMF) conditions. Effect of transparent PET bottles, and an alternative opaque packaging was assessed. Both types of bottles were sealed under nitrogen. Peroxide and retinol parameters were compliant with the national regulations for safety and fortification at all points in the supply chain, regardless of exposure to light. However, after 40 days use (i.e., SMF-condition⁵) in households did not meet safety and fortification regulations. The major inducer of oxidative deterioration and vitamin A losses was the daily opening of bottles for household use, regardless of exposure to light. Households with five or fewer members are recommended to buy 1-liter bottles rather than 5-liter bottles of oil to reduce time of use after the opening of the bottle.

Keywords: sunflower oil, fortification, peroxide value, vitamin A stability, retinol retention, supply chain

Introduction

In Kazakhstan, the prevalence of vitamin A deficiency (VAD), assessed through low serum retinol concentration (< 0.7 µmol/L), is higher than 20% in children under five years of age with a marked seasonal difference, varying from 56% of children in the spring and 23% of children in the autumn [1-3]. This situation identifies a severe public health problem according to World Health Organization (WHO) criteria.^[4] Vitamin D deficiency (VDD) is also a public health problem in Kazakhstan, owing to its geographical location (beyond parallel 35). In countries located at similar latitude as Kazakhstan, VDD ranges from 30% to 66% from November to March where the synthesis of vitamin D in the skin is low in the northern hemisphere.^[5] A recent article reported that 34.6% women and 16.7% males had 25-hydroxy-vitamin D concentrations in plasma lower than 10 ng/mL in Kazakhstan^[6], and which is slightly lower than the threshold of 12 ng/mL (30 nmol/L) that has been recommended to diagnose vitamin D deficiency in populations.^[7] This situation reveals that vitamin D deficiency in Kazakhstan as a public health problem, specially in women. According to early studies in 1973, 62% of infants have rickets when virtually all children received vitamin D preparation.^[8] Large-scale food fortification with vitamins A and D might be a cost-effective and long-term strategy for prevention of deficiencies of these two vitamins.^[9-11] as of today, 36 countries have standards to fortify edible oil with vitamin A and/or vitamin D.^[12]

Vegetable oil intake in Kazakhstan is estimated at 50.6 g/day per person.^[13] The main type of oil consumed nationwide is sunflower oil (77.7%). Other oils are consumed to a lesser extent: 11.6% of palm oil, 4.1% of rapeseed oil, 3.2% of linseed oil, 1.4% of soybean oil, 0.7% of cottonseed oil, and 1.1% of other oils.^[14] Sunflower oil is packaged by weight or volume in containers made of materials permitted for contact with vegetable oils in accordance with the established practices. Consumer containers with sunflower oil are hermetically sealed with caps, stoppers or lids made of materials permitted for contact with vegetable oils.^[15] In Kazakhstan and in the countries of the Eurasian Economic Union (EAEU) and in the Commonwealth of Independent States (CIS), the most common option is packaging of refined sunflower oil in plastic bottles with two-composite plastic caps under a nitrogen cushion. Standard 2-component lid O28 is suitable for completing packaging for sunflower oil, vinegar and other liquids. It is a highly effective barrier closure for the neck of plastic bottles. It is made of high-density polyethylene (HDPE, upper part oil cap: 28 mm, usually yellow, less often green) and low-density polyethylene (LDPE, lower part, usually white), due to which the high strength of

the material is achieved. The two-piece cap provides convenience and safety for consumers. The device is equipped with protection against unauthorized access, which guarantees reliable identification of the first opening. The identification ring that remains on the bottle provides not only protection for the product, but also consumer confidence in the authenticity of the product. For delivery to the market, bottles are packed in aggregate opaque cardboard pack, each containing 15 bottles.

A recent assessment of the oil industry and supply chain of sunflower oil identified two types of oil factories in Kazakhstan: Large factories (LF) which process 1,000 or more tons of sunflower seeds per day, and medium/small factories (SMF) which process several hundred tons of seeds per day. There are two large factories in the country, having 55% or more of the total market share, and 7 or 8 SMF contributing to 25 to 30% of the total oil market. The remaining oil is imported from the Russian Federation and Ukraine.^[16, 17]

The Kazakh Academy of Nutrition (KAN) has been advocating for the fortification of vegetable oils as an appropriate strategy to control vitamin A and D deficiencies in Kazakhstan. As part of these efforts, KAN developed the *National Standard ST RK 3547-2020 "Vitaminized sunflower oil. Technical regulations"*,^[18] which has been adopted and came into force in January 2021 (Order No. 283-od of the Committee for Technical Regulation and Metrology of the Ministry of Trade and Integration of the Republic of Kazakhstan dated August 5, 2020). This standard specifies the addition of a mean content of 18.0 mg/kg for vitamin A, and 0.15 mg/kg for vitamin D.

Vitamin A requires to be added to a very high-quality oil and be protected of exposure to light, oxygen, and high temperatures. Vitamin A is particularly sensitive to peroxides, one of the primary by-products of oil oxidation. The initial quantity of peroxides in oil at the manufacturing and bottling phase has a strong influence on the rate of vitamin A decomposition and overall shelf life.^[19]

The objectives of this study were to evaluate the stability of vitamin A in the oil under the conditions of marketing of LF and SMF along the market chain. We tested two alternative packaging materials (current transparent and opaque) on the shelf-life of this vitamin. We measured the effect of storage time, and exposure to light on the concentrations of peroxides and vitamin A.

Materials and Methods

This study mimicked in the laboratory real-life conditions of factors that may affect the stability of vitamin A in sunflower oil along the entire supply chain. The changes in peroxide value (PV) and the retinol retention were assessed. Additionally, the study also tested two different packaging options: the current transparent (Tr) polyethylene terephthalate (PET) polymeric bottle, and an alternative opaque (Op) bottle packaging.

Study design

The study was designed based on results of the assessment of the oil industry and supply chain of sunflower oil, which are presented in Table 1.

Table 1: Storage, transportation and light exposure days at key time-points of the two types of oil factories and supply chain conditions

Key time-points of supply chain	LF	SMF
Baseline production day	0 day	0 day
Bottling time (filling and closing)	1 minute	10 minutes
Time of storage in factory warehouse in cardboard boxes before going to market (Dark)	2 days (2D)	10 days (10D)
Time of delivery from factory to retailers' warehouse in cardboard boxes (Dark)	10 days (12D)	20 days (30D)
Time of storage in retail shops in cardboard boxes (Dark)	5 days (17D)	10 days (40D)
Time of exposure at retailers 'shelves (Light)	5 days (17D+5L)	10 days (40D + 10L)
Average turnover at the market end	22 days (17D + 5L)	50 days (40D + 10L)
A. Time of storage/use at households after opening the bottle cap (always kept in the dark)	20 days (37D + 5L = 42 days)	40 days (80D + 10L = 90 days)
B. Time of storage/use at households after opening the bottle cap with access to light	20 days (17D + 25L = 42 days)	40 days (40D + 50L = 90 days)

LF: Large factory; SMF: Small-to-medium factory; Numbers in parenthesis = Days of accumulated storage after production, in dark (D) or exposed to light (L).

At the retail and household levels, two alternative conditions were studied after opening the oil container: keeping it in the dark or exposed to light (see the two last lanes of Table 1). We assumed that product from the LF was consumed up to 42 days, and that of SMF up to 90 days. This because of the more duration of each stages in supply chain of product from SMF than that of LF product. The largest exposure to light was at households using SMF product (40 days against 20 days of LF product). Here, it is very important to point out that the oil was produced by only one large factory; only the conditions of storage/exposure for LS and SMF were different.

Experimental fortification trial

Sunflower oil is currently not fortified in Kazakhstan. A pilot batch of fortified sunflower oil was produced at the Eurasian Foods Corporation (EFC) oil plant in Almaty. This follows the standard process of sunflower oil production in large factories.

The pilot batch consisted of mixing unfortified sunflower oil with vitamins A and D premix in order to obtain 500 kg of 'Vitaminized (fortified) Sunflower Oil' (VSO). Vitamin premix "Oily Vitamin Blend 02-185" was obtained from BASF as an oil mixture of vitamin A (retinyl palmitate) with an activity of 1,000,000 IU/g and vitamin D₃ (cholecalciferol) with an activity of 100,000 IU/g. Gross composition of the premix is as follows: vitamin A palmitate is 55–60%; vitamin D₃ is 0, 25-0,275 % and sunflower oil up to 100%. The rate of addition of vitamin premix for fortification is 60 g/ton of unfortified oil. However, due to the high concentration of vitamins A and D in the premix, a pre-mixture was first prepared by diluting the premix with refined deodorized sunflower oil at the ratio of 1:100. The premix taken out from the cold store was heated to approximately 40 - 45 °C before mixing. This is to ensure that there are no vitamin A crystals in the sample as vitamin A tends to crystallize during cold storage.

After thorough mixing, the mobile tank with 500 kg content was moved to the filling machine and fortified sunflower oil was automatically bottled in 1-liter polyethylene (PET) bottles and sealed under nitrogen. Ninety bottles with VSO were sampled for research. For testing different packaging, the 90 bottles were divided into 2 groups: a group of 45 bottles remained in the usual transparent packaging (PET) and put in 3 cardboard boxes; while a group of 45 bottles were manually placed in individual lightproof bags and the neck of the bag was fixed with an elastic band, and then placed again in cardboard boxes. Boxes of VSO bottles were identified (encoded). The printed codes were prepared in advance, and attached to bottles with previously prepared adhesive tape. These codes identified the quality of transparent (Tr) or opaque (Op), and the number of replicates.

Baseline value at time zero and along the supply chain

The values of the two parameters (vitamin A and peroxide contents) were measured at time zero, just after bottling, and they were considered as the baseline values. Values at each point of the supply chain were compared to the baseline value and statistical significance of the difference between the two values was estimated. At each step of the supply chain, 3 samples were taken, and for each sample, two analyses were run for each parameter in the lab. Therefore, results at each point are mean values of the 6 analyses.

Temperature

The temperature fluctuated between 20 °C and 25 °C year-round at all points of the supply chain. This was the ambient temperature in Almaty where the study was done.

Light exposure

Oil bottles that were not removed from cardboard boxes were always considered as stored in the dark. Only when bottles were taken out of the cardboard box and put on shelves in retail stores or in household kitchen were considered as exposed to light.

Sampling and sample delivery to the lab

Table 2 summarizes the procedure for sampling of VSO. At each step of the supply chain, three samples were taken, and for each sample, two analyses were run for each parameter in the lab. When the results were outside the expected variation ($\geq 5\%$), the analyses were repeated. In total, we analyzed 42 samples for each type of condition, and up to 200 chemical analyses of peroxides and vitamin A were completed.

The bottles were stored for 30 days in a separate place in the EFC warehouse with a separate pallet with identification marks prohibiting the use of experimental oil for trade under the responsibility of the warehouse manager. They were delivered from the factory to KAN "Nutritest" laboratory in separate cardboard boxes at the times indicated by the protocol designed purposely for "Nutritest" Laboratory analysis.

Table 2: Sampling procedure and sample calculation

Steps in supply chain (Impact factors)	Large factory		Small-to-medium factory	
	Exposure days	Sample number	Exposure days	Sample number
At production (Zero day at factory)				
- VSO in Tr (1 minute)	--	3		
- VSO in Opaque (1 minute)	--	3		
- VSO in Tr (10 minutes)			--	3
- VSO in Opaque (10 minutes)			--	3
Storage in factory in Dark				
- VSO in Tr bottles	2	3	10	3
- VSO in Opaque	2	3	10	3
Delivery from factory to retailers' shops in Dark				
- VSO in Tr bottles	10	3	20	3

- VSO in Opaque	10	3	20	3
Storage in retailers' shops in Dark				
- VSO in Tr bottles	5	3	10	3
- VSO in Opaque	5	3	10	3
Exposure in retailers' shops in Light				
- VSO in Tr bottles	5	3	10	3
- VSO in Opaque	5	3	10	3
Storage and use in households after intermittent opening bottle cap in Dark				
- VSO in Tr bottles	20	3	40	3
- VSO in Opaque	20	3	40	3
Storage and use in households after intermittent opening bottle cap in Light				
- VSO in Tr bottles	20	3	40	3
- VSO in Opaque	20	3	40	3
Total days of exposure/samples	42	42	90	42
Total samples analyzed	84 samples (168 analyses) + 20% for losses (32) = 200 analyses			

Lab analysis

The measurement of the peroxide value in fortified sunflower oil was carried out in accordance with the Interstate Standard GOST 26593-85.^[20] The method is based on the interaction of the oxidation products of vegetable oils and fats (peroxides and hydroperoxides) with potassium iodide in a solution of acetic acid and chloroform, and the subsequent quantitative determination of the released iodine with a solution of sodium thiosulfate by the titrimetric method. Peroxide value (PV) of vegetable oil was expressed millimole of active oxygen per kg of oil (mmol ½ O), which is equivalent to "milli-equivalents" (meq) of active oxygen per kg of oil (meq/kg).^[21]

Codex Alimentarius (CODEX-STAN210 – 1999) sets the quality characteristics for PV in refined oils, including refined sunflower oil, as up to 10 meq/kg of active oxygen/kg oil and up to 15 meq in cold pressed oils or virgin oils.^[22] In Kazakhstan, the Technical Regulations of the Customs Union TR CU 024/2011 "Technical Regulations for Fat and Oil Products", also set the peroxide value in all types and fractions of vegetable oils to be no more than 10.0 meq/kg ½ O.^[23]

The content of total trans-retinol and 13-cis-retinol was measured in fortified sunflower oil by High Performance Liquid Chromatography according to Interstate Standard GOST EN 12823-1-2014.^[24] Analysis was performed according to a protocol prepared by KAN, and briefly summarized in the Materials and Methods section. Biochemical analyses at "Nutritest" Laboratory begin on the day of oil delivery from the factory.

Results

Content of peroxide and retinol in sunflower fortified oil in transparent packaging (Tr)

Mean peroxide value

Table 3 shows mean peroxide values (PV) of oil along the entire supply chain of the two types of supply chain conditions (LF and SMF). PV values were lowest at production (baseline), and were significantly higher at all time points thereafter (P<0.000). Oil under SMF supply chain condition showed higher PV values at almost all time points, except at baseline, and these was associated to the larger number of days after production.

Table 3: Mean Peroxide Value (meq/kg) by type of oil factory supply chain condition (LF vs. SMF) in transparent packaging

Steps in supply chain from production to households	Days after production	Supply chain		P-values against the baselines	
		LF	SMF	LF	SMF
Baseline value	0	0.76	0.74		
Factory storage	2	0.81	-	0,111	-
Factory storage	10	-	1.10	-	0,002
Delivery to retailer warehouse	12	1.08	-	0,000	-
Retailer storage	17	1.17	-	0,000	-
Retailors' s shelves (Light)	5L-22	1.44	-	0,009	-
Delivery to retailer warehouse	30	-	1.61	-	0,000
Retailer storage	40	-	1.72	-	0,000
Retailors' s shelves (Light)	10L-50	-	1.75	-	0,000
Value at household (Dark, and opened)	20L-42	3.67	-	0,000	-
Value at household (Light, and opened)	20L-42	4.48	-	0,000	-

Value at household (Dark and opened)	40L-90	-	13.39	-	0,000
Value at household (Light, and opened)	40L-90	-	12.98	-	0,000

In all cases, oil was in a dark condition, except when indicated as (Light or L).

Mean PV remained within the acceptable level (less than 2 meq/kg) up to the point of sale (i.e., after production, factory storage, supply to retailers, retail storage and up to retail shelves), and which until this point were under dark (hardboard boxes). PV increased - but remained under the value of 2 meq/kg - when oil bottles were taken out of the cardboard box and exposed to light at the retail shelves for both types of supply chain conditions. Nevertheless, oil subjected to SMF conditions showed larger PV because of the additional number of days after production.

However, an important increase in PV occurred at household level for both supply chains when the bottles of oil were opened. Protection against light exposure did not reduce the formation of peroxides. In the oil under LF supply chain condition (after 42 days of production and 15 days after opening of the bottle), the PV was still lower than the threshold of 10 meq/kg. In the oil treated under SMF condition, the PV increases above that threshold after 40 days of production. This can simply be explained by the larger number of the days after opening the bottle (30 days) in the SMF condition. The increased PV beyond 10 meq/kg makes oil after around 20 days of being opened is unfit for human consumption according to Kazakhstan technical regulations. Whether this increment is associated to the presence of vitamins in the oil or not is unknown at this moment, as we did not include controls without fortification.

In summary, the big difference between the two supply chain conditions is at homes, after the opening of the bottles. At homes, preventing against light exposure does not have an influence in the production of peroxides, it is the opening of the bottle the main factor. Obviously, in the case of SMF condition, the production of peroxides is larger because the time of exposure is longer.

We conclude that oil quality remains suitable for human consumption for up to 50 days, or perhaps longer, if the bottles remain closed, regardless of the exposure to light for 5 to 10 days at the retail stores. The peroxide content increases beyond standards between 20 and 30 days of the opened oil bottles at households, and this is independent from light exposure.

Mean retinol content

Mean retinol concentrations at baseline were similar between LF and SMF supply chain conditions, and it declined over time for both (Table 4). However, the decline in retinol concentrations accelerated after the bottles were opened, and the decay was larger under the SMF conditions because of the longer period of time after opening. As in the case of the formation of peroxides, protection against exposure to light did not have impact.

Table 4: Mean Retinol content (mg/kg) by type of oil factory supply chain conditions (LF vs. SMF) in transparent packaging

Steps in supply chain from production to households	Days after production	Supply chain		P-values against the baselines	
		LF	SMF	LF	SMF
Baseline value	0	18.8	18.6		
Factory storage	2	18.5	-	0,272	-
Factory storage	10	-	18.1	-	0,212
Delivery to retailer warehouse	12	18.0	-	0,037	-
Retailer storage	17	17.8	-	0,021	-
Retailers' s shelves (Light)	5L-22	17.2	-	0,011	-
Delivery to retailer warehouse	30	-	16.7	-	0,013
Retailer storage	40	-	15.9	-	0,005
Retailers' s shelves (Light)	10L-50	-	16.6	-	0,008
Value at household (Dark, and opened)	20L-42	15.1	-	0,002	-
Value at household (Light, and opened)	20L-42	15.2	-	0,001	-
Value at household (Dark and opened)	40L-90	-	10.2	-	0,000
Value at household (Light, and opened)	40L-90	-	10.0	-	0,000

In all cases, oil was in a dark condition, except with indicated as (Light or L).

Under dark conditions, the mean retinol content decreased slightly (5%) from factory to retailer's delivery and storage after 17 days, and a little more but still acceptable (15%) after 40 days of production. At home level, after 42 days of production, and 15 days after opening of the bottle (LF conditions), vitamin A decay was 20%, and half of those from the moment of opening of the bottle. However, decay after 90 days of production, and 30 days of opening of the bottle (SMF conditions), the decays was 46%, and half of that after opening of the bottle. These results confirmed that the main reason of vitamin A decay is the opening of the bottle.

This marked difference in retinol content between the two types of supply chain oils at household level mirrors the same difference in mean peroxide value observed earlier. Therefore, this is suggestive that the retinol loss is a direct result of the content of peroxides and not of exposure to light. According to general recommendations, fortified oil with a retinol retention rate below 70% at the consumer level is not an optimal practice, and which

means that once a bottle of oil is opened, the fortified oil should be consumed within 20 days, both for keeping the vitamin A content as well as for not providing excessive amounts of peroxides.

In conclusion, fortification of oil in Kazakhstan, using the conditions of production, distribution and marketing of the fortified oil produced by the industry that collaborated in this study are acceptable because both peroxide value and retinol content are within the regulatory and recommended limits across the entire supply chain up to the consumer plate. However, once the oil bottle is opened, oil should be consumed within 20 days, because after this period the formation of peroxides and the associated loss of retinol accelerate. Our results suggest that the main reason of the retinol losses in oil is the content of peroxides and not the exposure to light.

However, the design of our study did not allow a comparison with the oil actually produced by SMF factories, and whose peroxide content at baseline may be different to the oil produced by the LF that was involved in this study.

Oil quality (PV) and retinol values in transparent and opaque packaging

Tables 5 and 6 show the equivalent results to Tables 3 and 4, respectively, but applied to oil samples that are protected from light exposure inside opaque containers along the supply chain and household use.

Peroxide value using opaque containers

Results of PV in oil stored in opaque containers reflect the same pattern as in transparent containers (stored in dark or light environments). However, the values were lower up to the retailer's shelves, and which means that packaging the SFO in opaque containers reduce the formation of peroxides (around 40% lower of the values at 22 and 50 days after production) when the bottles have not been opened. However, once the oil bottles are opened, the amounts of peroxides that are formed are similar than in the PEC bottles. These findings corroborate the conclusion that the main reason of the formation of peroxides is the exposure of the oil to the environment after opening the bottle. In summary, this finding indicates that exposure to atmospheric oxygen takes a predominant role in the oxidative process over light exposure at the bottle opening, and therefore opaque packaging, although reduces peroxide formation in closed bottles, has little influence in the PV once the bottles of oil are opened.

Table 5: Mean Peroxide Value (meq/kg) by type of oil factory supply chain condition (LF vs. SMF) in opaque containers

Steps in supply chain from production to households	Days after production	Supply chain		P-values against the baselines	
		LF	SMF	LF	SMF
Baseline value	0	0.73	0.71		
Factory storage	2	0.80	-	0,052	-
Factory storage	10	-	0.82	-	0,016
Delivery to retailer warehouse	12	0.82	-	0,138	-
Retailer storage	17	0.80	-	0,215	-
Retailors' s shelves (Light)	5L-22	0.88	-	0,005	-
Delivery to retailer warehouse	30	-	1.10	-	0,000
Retailer storage	40	-	1.11	-	0,000
Retailors' s shelves (Light)	10L-50	-	1.16	-	0,000
Value at household (Dark, and opened)	20L-42	3.62	-	-	-
Value at household (Light, and opened)	20L-42	4.15	-	-	-
Value at household (Dark and opened)	40L-90	-	12.92	0,000	0,000
Value at household (Light, and opened)	40L-90	-	13.29	0,006	0,000

In all cases, oil was in a dark condition, except with indicated as (Light or L).

Retinol retention using opaque containers

In the case of retention of vitamin A, the opaque container reduces decay once the bottles are opened but imperceptible when the bottles are close. This suggests that an opaque container reduces the vitamin A loss caused by the peroxides. However, the difference may not be so significative for promoting the change of the type of container from PEC to opaque bottles; the most important factor is to reduce the formation of peroxides, and those are reduced by limiting exposure of the fortified oil to air.

Table 6: Mean Retinol content (mg/kg) by oil factory supply chain condition (LF vs. SMF) in opaque containers

Steps in supply chain from production to households	Days after production	Supply chain		P-values against the baselines	
		LF	SMF	LF	SMF
Baseline value	0	18.5	18.4		
Factory storage	2	18.6	-	0,530	-
Factory storage	10	-	18.6	-	0,618

Delivery to retailer warehouse	12	18.4	-	0,530	-
Retailer storage	17	18.1	-	0,172	-
Retailers' s shelves (Light)	5L-22	17.7	-	0,004	-
Delivery to retailer warehouse	30	-	17.6	-	0,085
Retailer storage	40	-	17.1	-	0,055
Retailers' s shelves (Light)	10L-50	-	17.2	-	0,008
Value at household (Dark, and opened)	20L-42	15.9	-	0,001	-
Value at household (Light, and opened)	20L-42	15.8	-	0,001	-
Value at household (Dark and opened)	40L-90	-	11.2	-	0,000
Value at household (Light, and opened)	40L-90	-	13.0	-	0,000

In all cases, oil was in a dark condition, except with indicated as (Light or L).

An important question to respond is what concentration of peroxides is determinant to expect an unacceptable decay of vitamin A? And another related to this is how so efficient is the reduction of peroxides by using antioxidants. In other words, can the data show a dose-response relationship between these two parameters, or a peroxide threshold value at which retinol retention is going to be highly affected? Our results do not allow for estimating this relationship adequately mathematically because of the absence multiple data points or a data series linking changes in PV and retinol content. Instead, we evaluated this relationship linearly based on the values of PV and retinol retention at two typical points of the supply chain: end of market supply chain, and at households, under light exposure. Table 7 compares the increase of PV and decrease of retinol retention at these two supply chain points for both supply chain conditions (SMF and LF) by packaging type (Tr and Op).

Table 7: Comparing percent increase of PV and percent decrease of retinol retention in oil depending on packaging type and the duration of market supply chain (M-SC) and household use (HH-use)

Characteristics	LF/Transparent		LF/Opaque		SMF/Transparent		SMF/Opaque	
	M-SC, 22 days	HH-use, 20 days	M-SC, 22 days	HH-use, 20 days	M-SC, 50 days	HH-use, 40 days	M-SC, 50 days	HH-use, 40 days
% PV	+94.6	+505.4	+23.9	+484.5	+136.5	+1654	+63.4	+1772
% Retinol content	-8.5	-19.1	-4.3	-14.6	-10.8	-46.2	-6.5	-29.3
Percent Increase: PV: HH-use/M-SC ^{A)}	5.3		20.3		12.1		27.9	
Percent Decrease: Retinol content: HH-use/M-SC ^{B)}	2.2		3.4		4.3		4.5	
^{A)/B)}	2.4		6.0		2.8		6.2	

Under transparent packaging and light exposure, comparison of PV and retinol content ratio (A/B) at the two supply chain points (HH-use and M-SC) shows almost the same proportion in oil of both supply chain conditions (SMF/Tr and LF/Tr), that is 2.4 to 2.8. We can safely state that a unit change in retinol content under these conditions is associated with a 2.4 to 2.8 change in PV.

On the other hand, under opaque packaging and light exposure, the same comparison of PV and retinol content ratio at the two supply chain points (HH-use and M-SC) shows almost the same proportion in oil of both supply chain conditions (SMF/Op and LF/Op), that is 6.0 to 6.2. Similarly, this means that a unit change in retinol content under these conditions is associated with a 6.0 to 6.2 change in PV.

We conclude that PV and retinol content variations are inversely proportional (negative sign in retinol content), and the relationship is seemingly of a dose-response nature varying with the packaging type. A decrease of retinol content by 1 unit is associated with a two-to-three times increase in PV in transparent bottles, and a six-time increase of PV in opaque bottles. A simple interpretation of this finding is that opaque packaging might have a de-escalation effect on vitamin A degradation as compared to transparent packaging.

The impact of opaque packaging on retinol content and peroxide value in LF and SMF oil

Table 8 shows data on the comparative effect of transparent and opaque packaging on the peroxide value and retinol content in fortified sunflower oil when stored in the light on retail's shelves and in households after opening the bottles and daily use of oil. These data indicate some protective effect of opaque packaging against the degradation of retinol in LF and SMF oil when simulated under light storage on retail's shelves and in households compared to the corresponding levels of retinol in LF and SMF oil in transparent bottles. A similar protective effect was found in relation to the protection against excessive formation of peroxides in LF and SMF oil until opening the bottle cap in households. However, after 40 days of opening the bottle at households, the protective effect of opaque packaging from the formation of peroxides was not revealed. This may be due to the increased availability of oxygen for the formation of peroxide during daily bottle opening and oil use.

Table 8: Comparative impact of transparent bottles and opaque packaging on peroxide value and retinol content in fortified sunflower oil when stored under light

Parameter	Peroxide (meq/kg)		Retinol (mg/kg)		Peroxide (proportion)		Retinol (proportion)	
	Tr	Opaque	Tr	Opaque	Tr	Opaque	Tr	Opaque
Type of packaging								
Baseline	0,76	0,73	18,7	18,5	1,00	1,00	1,00	1,00
5L-22 days (LF): Retailers' shelves (light)	1,44	0,88	17,2	17,7	1,92	1,22	0,92	0,96
10L-50 days (SMF): Retailers' s shelves (light)	1,75	1,16	16,6	17,2	2,33	1,61	0,89	0,93
20L-42 days (LF): Value at household (light, and opened bottles)	4,48	4,15	15,2	15,8	5,97	5,76	0,81	0,86
40L-90 days (SMF): Value at household (light, and opened bottles)	12,98	13,29	10,0	13,0	17,31	18,46	0,53	0,70

Potential alternative options to opaque packaging in SMF oil

These results show that opaque packaging is not the right option for an effective protection of fortified oil under SMF supply chain conditions. The determining factor for lower vitamin A retention under SMF supply condition is the formation of peroxides, and which are directly associated to the frequency and duration of intermittent exposure to atmospheric oxygen once the bottle caps are opened (>20 days). Alternative options should be found to reduce the content of peroxides, by decreasing the time of exposure to oxygen of opened bottles at the household level. The other alternative, and which we did not assess, was the effective use of antioxidants in the oil for preventing the formation of peroxides.

In the case of Kazakhstan, the immediate question to be raised by this finding is “how to reduce the time of use of ‘SMF oil’ at household level, and what other effective protection measures can be taken in order to decrease the effect of exposure to oxygen?”

The time of domestic use of oil depends on the quantity of oil at each purchase, and the size of the household purchasing it. Sunflower oil comes predominantly in two sizes in Kazakhstan: 1-liter and 5-liter bottles, with currently a larger proportion of production of 5-liter bottles, that is, 70% against 30% (14). Calculation of average number of days of oil consumption with 1-liter and 5-liter bottle purchase, depending on the national statistics on household size in Kazakhstan, ^[25] is presented in Table 9. The average household size in Kazakhstan is 3.6 people, and the average oil consumption is about 40 ml/ day per capita. Given these figures, the calculated average number of days for the consumption of 1-liter oil bottle by an average household in the country is 6.9 days. The largest average number of days for consumption of 1-liter of oil falls in a household consisting of 1 person (25 days) or 2 persons (12.5 persons).

Table 9: Calculation of average number of days of oil consumption with 1-liter and 5-liter purchase depending on size of households (HH), based on 2009 Population Census data ^[25]

Average HH size		Number of HH	% of HH	Average oil consumption, ml per capita/ day	Average number of days for consumption of 1-liter of oil	Average number of days for consumption of 5-liter of oil
1	person	639890	14,6	40	25,0	125,0
2	persons	834846	19,0	40	12,5	62,5
3	persons	848198	19,3	40	8,3	41,7
4	persons	825198	18,8	40	6,3	31,3
5	persons	535009	12,2	40	5,0	25,0
6	persons	349605	8,0	40	4,2	20,8
7	persons	160957	3,7	40	3,6	17,9
8	persons	86192	2,0	40	3,1	15,6
9	persons	46302	1,1	40	2,8	13,9
10+	persons	65562	1,5	40	2,5	12,5
All HH	3,6 persons	4391759	100	40	6,9	34,7

The oil industry assessment ^[16] reported an average duration of 40 days at household for oil delivered through SMF supply chain condition. This is twice the average duration of 20 days for “LF oil”. This implies that most households using ‘SMF oil’ are purchasing 5-liter bottles. This finding is very likely to be true because small and medium size oil factories are more oriented towards rural areas where oil turnover in the market is slower than in large cities.

Households can buy oil in a 5-liter bottle if the family consists of more than 5 people, but families with fewer people are better off buying oil in a 1-liter bottle.

From the industry perspective, the implementation of this option may not cause significant financial losses for oil producers since the cost of one 5-liter bottle oil is equal to the cost of five 1-liter bottles oil due to the high cost

of packaging material and packaging process for 5-liter bottle oil. However, this statement should be validated through direct consultation with the oil industry.

Our study did not assess the effectiveness that allowable antioxidants can reduce the formation of peroxides in oil exposure to air. Moreover, it is also important to determine the baseline content of peroxides in oil manufactured by small-to-medium factories and to follow its increment along the distribution and marketing chain. These are important parameter that deserves to be measured to establish the requirements for using oil as an appropriate vehicle for fortification of vitamins A and D.

Discussion

To our knowledge, this paper is the first to investigate the quality and stability of vitamin A in fortified sunflower oil along the market chain in Kazakhstan. In this paper, we show significant differences in the content of peroxides and vitamin A retention in oil delivered to consumers, once the bottles are opened. The difference for a good quality product and program depends on the duration of use of the oil at households. As small-to-medium size factories prefer to package their product in large bottles (5 L), this is a limiting factor that decreases the supply of vitamin A, and perhaps also vitamin D, to the population through fortified sunflower oil.

It is known that the quality of vegetable oil, assessed by the PV before fortification has a strong effect on the stability of retinyl palmitate, ^[19, 26] and the higher the oxidative values, the lower the retention of vitamin A overtime. ^[27-30] In line with these findings, our study shows that as PV increases across the supply chain retinol retention decreases simultaneously, even though opaque packaging reduces the formation of peroxides when the bottles are not opened. However, in our study, contrary to the result of the Egypt study, ^[19] a low PV value of less than 2 meq/kg at the production site did not guarantee an acceptable stability of vitamin A at the household level. We explain this finding because the formation of peroxides once the oil bottles are opened, and which is important beyond 20 days. Moreover, opaque containers protected in slight degree the decay of vitamin A in opened bottles, but the improvement was too modest as to promote the use of this type of packaging for FSO. The main factor to increase the retention of vitamin A in oil is to reduce the formation of peroxides.

Our study did not assess the effect of cooking (temperature) on the stability of vitamin A in fortified oil. Many published studies indicate that only minor losses are incurred during normal cooking procedures. ^[31-35]

Variations of PV can arise from different factors including the degree of saturation of fatty acids present in the particular oil, temperature, storage, exposure to light, oxygen (air), and the content of metals and other compounds that may catalyze the oxidation processes. ^[30] This study assessed the influence on oil quality of only time of storage, exposure to light and exposure to oxygen, while temperature was kept at a constant room level (20°C - 25°C), the temperature commonly found during the oil industry assessment in Kazakhstan. Our study confirms findings in earlier studies on sunflower oil showing a steady PV increase with time whether oil is daylight exposed or kept in the dark, ^[26, 36] but our results demonstrate that the critical moment is when the oil is exposed to air, before that moment in a well closed package, including transparent bottles, the formation of peroxides is slow and therefore the retention of vitamin A is favored

A particular aspect of this study is the evidence that both peroxide formation and vitamin A retention is more influenced by intermittent exposure to atmospheric oxygen (air), than by exposure to light, particularly at exposure time exceeding 20 days when bottles are open.

Finally, to cope with the marked increase of peroxide value and the low retinol retention under SMF oil supply condition with long periods of use at households, the use of additive antioxidants may be one alternative. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl-hydroquinone (TBHQ) are popular among producers and have been shown to extend the shelf life of oils. ^[37] However, the low evidence of effectiveness and the cost of those measures are not in favor of this recommendation. First, while studies have shown the efficacy of antioxidants in extending the shelf life of oil, evidence on their effect on vitamin A specifically is limited. ^[34, 35, 37] Second, the cost of adding antioxidants in fortified oil or increasing the fortification contents might not be cost-effective in regard to the desired reduction of vitamin A losses. In summary, this topic deserves to be reviewed to be considered as an alternative to overcome the formation of peroxides, and the associated decay of vitamin A, in fortified oil that is used during a long period of time after being opened.

Conclusions

The key recommendations derived from our study for the oil industry, government and Kazakh Academy of Nutrition are

- Shifting to 1-liter bottle packaging to reduce the frequency and time of intermittent exposure of open bottles to atmospheric oxygen at the household.
- Generalizing the sealing with a tear-off-ring composite cap sealed under a nitrogen cushion to include oil produced by small-to-medium size factories.
- Determine the content of peroxide in oil manufactures by SMF.
- Work with the industry to determine the economical feasibility to switch only to the production of 1-L bottles.
- Reexamine the feasibility, cost and practical use of antioxidants in sunflower oil produced in the country.

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Author contributions

Conceptualization, MM and ST; Oil industry assessment: KN; Investigation: ST and OD; Lab analysis: OD; Data analysis: ST and MM; Writing: Original draft: ST; Re-writing: MM, ODr; Review and editing: DP, ODr. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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