

SIMULATING INTERNATIONAL SHIPMENTS OF VEGETABLE OILS: FOCUS ON QUALITY CHANGES

**Z. AYYAD^{1,2}, E. VALLI^{2,3}, A. BENDINI^{2,3}, R. ACCORSI¹, R. MANZINI⁴, M. BORTOLINI¹,
M. GAMBERI⁴ and T. GALLINA TOSCHI^{*2,3}**

¹Department of Food Technology, College of Science and Technology, Al-Quds University, Abu Dies, P.O. Box 20002, Jerusalem, Palestine

²Department of Agricultural and Food Sciences, Alma Mater Studiorum - University of Bologna, Cesena (FC), Italy

³Interdepartmental Centre for Agri-Food Industrial Research, Alma Mater Studiorum - University of Bologna, Cesena (FC), Italy

⁴Department of Industrial Engineering, Alma Mater Studiorum - University of Bologna, Italy

*Corresponding author. Tel.: +39 0512096010

E-mail address: tullia.gallinatoschi@unibo.it

ABSTRACT

This investigation evaluated the quality changes of commercial vegetable oils after different simulated shipments. In particular, the oils were placed in containers with or without thermal insulation and subjected to two simulated shipments, from Italy to Los Angeles and to Quebec. The temperature profiles were monitored to simulate the real shipments conditions in laboratory through properly developed climate chambers. Different quality parameters were evaluated before and after the simulations, showing a high degree of oxidation for samples shipped to Los Angeles in standard containers. In this study, the thermal insulation container was effective in protecting samples from potential oxidative damage during simulated shipping.

Keywords: edible vegetable oils, food quality, oxidation, simulated shipment; thermal insulation

1. INTRODUCTION

Vegetable oils such as sunflower, palm kernel, and soybean oils are extensively used for cooking purposes. These types of fatty food products are more susceptible to oxidation than animal fat because of their content of unsaturated fatty acids (PARKER *et al.*, 2003). In 2014, about 168 million tons of vegetable oil was produced worldwide (USDA, 2014). Among vegetable oils, Italy is considered as the dominant supplier of olive oils to Canada and USA, and about 72 % and 60 % of olive oil imported in 2014 to Canada and USA, respectively, was from Italy. Furthermore, in 2014, Italy exported around 230,000 metric tons of virgin olive oil (IOC, 2014b). During transportation by sea, the desired temperature for most edible oils is ambient temperature (CAC, 2013b). Considering that solidification and crystallization of the product occurs at 3-4°C (PISCOPO and POIANA, 2012), edible oils may suffer from deterioration in quality, which involve hydrolytic and oxidative modifications promoted by several factors, such as temperature and humidity in the stages of pumping and tank filling, in addition to the effect of light exposure for samples transported in clear bottles (BTM, 2013). Raw edible oils, even after soft refining, as well as virgin olive oils, contain a range of minor compounds such as chlorophylls, tocopherols, carotenoids, and phenolic compounds that function as natural antioxidants by enhancing the stability of the oil during storage (KRISTOTT, 2000). The monounsaturated/polyunsaturated fatty acid ratio, as well as the presence of phenolic compounds, make virgin olive oil more stable towards heat induced oxidation (BENDINI *et al.*, 2004). Moreover, the hydrolysis of acylglycerols, catalyzed mainly by an increase in temperature during storage, as well as the presence of moisture, oxygen, or light (FRANKEL, 1991), plays an important role in development of off-flavors, thus making edible oils unpalatable and shortening their shelf-life (KRISTOTT, 2000). High temperatures increase the rate of oxidation, while very low freezing temperatures may also change the availability of some micro components, such as phenolic compounds, water distribution around crystals, and the physical characteristics of olive oil (BENDINI *et al.*, 2007). Several studies have been carried out on the simulated transportation of foodstuffs. For example, an interesting report done by BURGER (1985) studied the effect of bulk storage and transportation on the quality of palm oil, and found that during the 25 days of an actual journey at temperatures ranging between 37-55°C, there was a slight increase in free acidity, while peroxide values were doubled at the final stage of the voyage. The effect of different thermal conditions registered in the food supply chain during transportation of edible oils was recently studied by our group (VALLI *et al.*, 2013). In that study, we investigated the effect of simulated shipment on the quality of different types of edible oils from Italy to Taiwan, starting from the stage of truck loading and ending at the truck delivery phase. It was found that vegetable oils underwent a loss of quality and deterioration after the journey, especially in terms of primary and secondary oxidation products. The simulation runs were conducted using ad-hoc closed-loop controlled chambers (MANZINI and ACCORSI, 2013), in order to measure and control the effects of transportation on the quality of edible oil. Moreover, we have also compared the performance of these containers (ACCORSI *et al.*, 2014; MANZINI *et al.*, 2014).

In the present study, changes in the quality of three kinds of vegetable oils (extra virgin olive oil, rice oil, and grape seed oil) after two simulated shipments were investigated. The first journey was characterized by high temperatures during 37 days of shipment from Italy to Los Angeles (USA), and the latter by lower temperatures during 30 days of shipment from Italy to Quebec (Canada). Such temperatures were monitored using a thermal data logger during actual shipping and then reproduced in the laboratory. In particular, both the shipping profiles experienced by the bottles were tracked within a standard container (SC), i.e., a general-purpose one or dry container, and a thermal liner

containers (TLC), i.e., a basic dry containers equipped with a thermal liner that can partially or completely insulate cargo from climate stresses. This study evaluated the ability of the thermal insulated container to protect the quality of the oils in both shipments. With this aim, quality parameters such as free acidity, oxidation indexes (peroxide value, thiobarbituric acid content, and oxidative stability index) as well as sensory analysis and other physicochemical parameters (water amount, turbidity, and CIElab color indexes) were evaluated before and after the simulated shipments.

2. MATERIALS AND METHODS

2.1. Samples

The two simulated shipments were carried out using three different kinds of commercial vegetable oils: extra virgin olive oil (EVOO), grape seed oil (GSO), and rice oil (RO). In particular, two bottles (1 liter each) of oil were subjected to the simulated shipments. The two bottles of each oil for each destination (Quebec, coded as "Q" or Los Angeles, coded as "LA") contained edible oil coming from the same production line batch.

For both bottles, the primary packaging is a glass bottle. The cork is made of aluminum with a PET pourer. The bottles are contained within a secondary package made by corrugated carton (i.e., wrap package). The shipping profiles experienced by the two sets of bottles are tracked respectively within a standard container (SC), i.e., a general-purpose 40-foot equivalent units (FEU) container or dry container, and thermal liner containers (TLC), i.e., a basic dry containers equipped with a thermal liner that can partially or completely insulate cargo from climate stresses. A more detailed definition of these two containers is given in ACCORSI *et al.* (2014), while for the primary (i.e., bottle) and secondary packaging (i.e., carton) (ACCORSI *et al.*, 2015).

2.2. Simulation process

The temperature profiles were reproduced using closed-loop climate-controlled chambers placed in standard or thermally insulated containers (Fig. 1). The two container solutions have been previously described in a paper by the same research group (MANZINI *et al.*, 2014). The simulation chambers reproduced temperature cycles to fit the monitored temperatures registered during actual shipments. The temperature inside the chambers covers the possible range of -20°C to 65°C. The integrated cooling system consists of an evaporator utilizing 21 g of R600a iso-butane as a refrigerant. A closed-loop algorithm, developed with LabView National Instrument software, controls the actuators so that the chamber temperature reaches a defined set point. The first international simulated shipment (coded as "Q") from Italy to Quebec started on January 30 from the port of origin (Livorno) and ended on March 1 at the port of final destination (Quebec); the temperature profile of this shipment is illustrated in Fig. 2. The second international shipment (coded as "LA") from Italy to Los Angeles started on June 26 from the port of origin (Livorno) and ended on August 2 at the port of final destination; the temperature profile of this shipment is shown in Fig. 3.

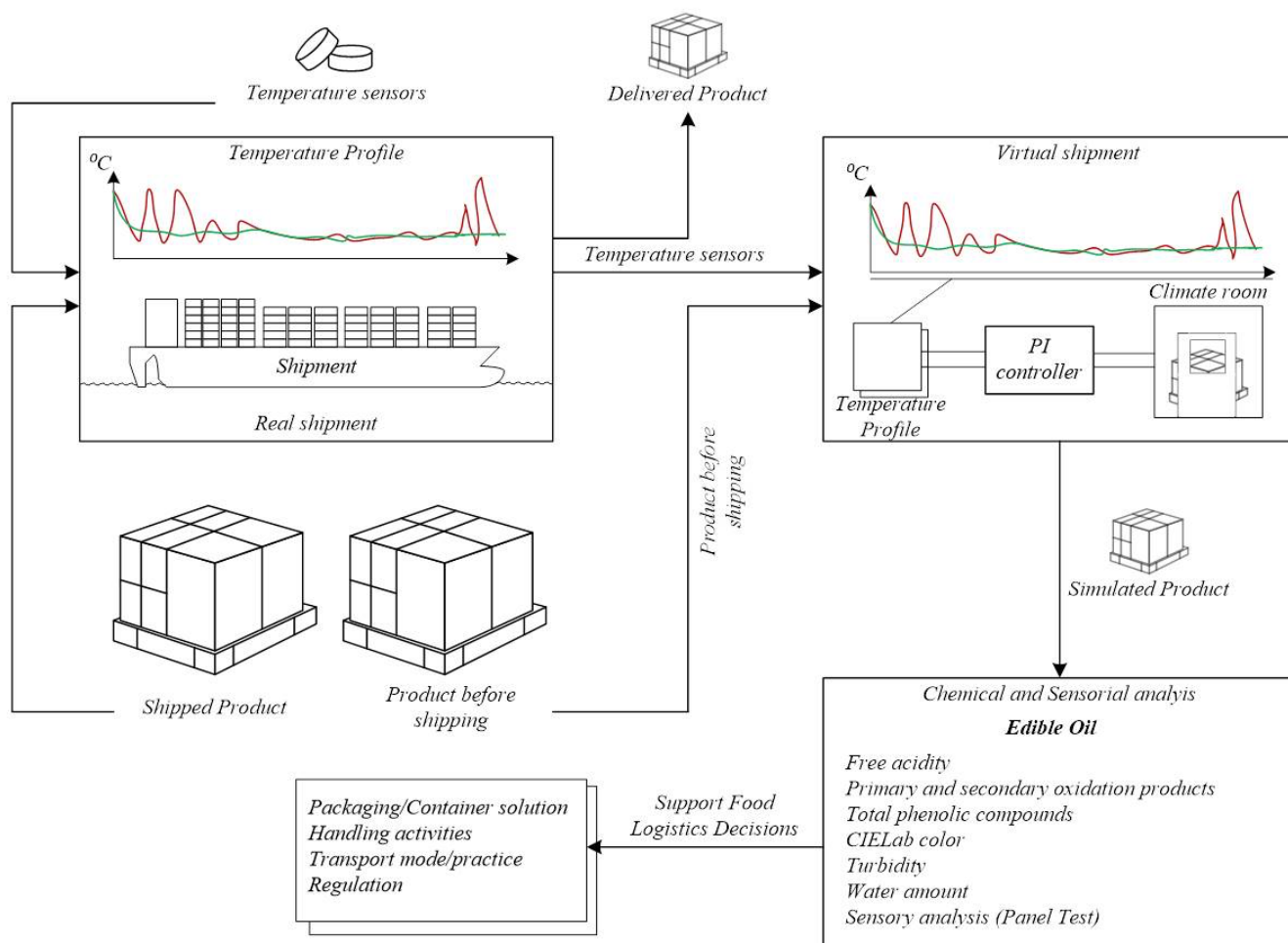


Figure 1. Closed-loop protocol system for simulation of shipping.

2.3. Chemical, physical and sensory analyses

2.3.1 Free acidity and thiobarbituric acid reactant substance content (TBARs)

Free acidity (FA) expressed as g oleic acid 100 g⁻¹ oil and peroxide value (PV) expressed as milliequivalent O₂ kg⁻¹ oil were determined for EVOO according to the official methods described in EEC. Reg. 2568/91 and successive amendments (EEC Reg. 1348/2013). For the two other edible oils, free acidity values (AV) were obtained by the Codex Alimentarius official method (CAC 2013a), and expressed in mg KOH g⁻¹ oil. Thiobarbituric acid reactant substance content (TBARs) was determined in triplicates according to the AOCS Official Method Cd 19-90 (AOCS, 2006) and expressed as TBA value (milligram of malonaldehyde equivalent per kilogram of oil). Oil sample (50-200 mg) was weighted into 25 ml volumetric flask and dissolved with a small portion of 1-butanol. The solution volume was then filled by using 1-butanol. A portion (5 ml) of the dissolved sample was transferred into a screw-capped test tube. The reagent solution (200 mg of 2-thiobarbituric acid dissolved in 100 ml of 1-butanol) was added, and the mixture was thoroughly mixed. The tubes were then placed in a water bath at 95°C for 2 h. After cooling at room temperature, absorbance was determined at 530 nm by using 1 ml glass

cuvettes with a UV-vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). The reagent blank was prepared simultaneous to sample preparation. TBA value was obtained using the following equation:

$$\text{TBA} = 50 \times (\text{abs of the sample} - \text{abs of the blank}) / \text{weight of the sample (mg)}$$

2.3.2. Spectrophotometric determination of total phenolic content (TP)

Phenolic compounds were extracted according to the method of PIRISI *et al.* (2000). Absorbance was determined at 750 nm by using a UV-vis 6705 spectrophotometer (Jenway, United Kingdom) through the method reported by Singleton and Rossi (1965). Briefly, each sample (2 g) was dissolved in 1 ml of *n*-hexane and extracted three times with 2 ml of methanol–water solution (60:40 v/v). In each extraction, the mixture was shaken with a vortex mixer for 1 min and then centrifuged for 5 min at 3,000 rpm. The aqueous phase was collected and transferred into another test tube after each centrifugation cycle. *n*-hexane (2 ml) was added to the collected phenolic extract, mixed on the vortex, and then centrifuged for 5 min at 3,000 rpm. After the *n*-hexane phase was removed, the extract was evaporated using a rotary evaporator at 35°C. The residue was dissolved with 5 ml of methanol-water solution (50:50 v/v). Absorption was determined with a spectrophotometer, and a standard calibration curve was prepared using different concentrations of gallic acid. The results were calculated and expressed as milligram of gallic acid per kilogram of oil.

2.3.3 Evaluation of the colour (CIELab)

CIELab color for EVOO samples was determined (GOMEZ-CARAVACA *et al.*, 2007), using a Hunterlab (Reston, VA, USA) colorflex instrument and expressed as L*, a*, b* chromatic coordinates. Turbidity (TD) of samples was determined using a Ratio turbidimeter model 18900 (Hack, Colorado, USA) and expressed as nephelometric turbidity units (NTU).

2.3.4 Determination of the water content

Water amount was determined at 103°C through air drying technique (ISO 662:1988). Oil sample (10 g) was weighed in an empty aluminum moisture dish (approximately 50 mm in diameter and 30 mm height, with a flat bottom). The samples were heated for 1 h in a drying oven at 103±2°C, and the dish was cooled in the desiccator and weighed. The sample was reheated for another 0.5 h, cooled, and then weighed again. The half-hour reheating, cooling, and weighing cycle may be repeated until the difference between the final successive weights was lower than 2 mg. The water amount was calculated with the following equation: weight of sample – weight of dried sample / weight of sample.

2.3.5 Sensory analysis

Sensory analysis of EVOO samples was performed according to the procedure outlined in EEC Reg. 640/2008 by a fully trained panel of 8 expert and trained tasters of the Department of Agricultural and Food Sciences of the University of Bologna.

2.3.6 Statistical analysis

All analyses were run in triplicate and expressed as mean \pm standard deviation (SD). Analysis of variance (ANOVA) was performed using XLSTAT 7.5.2 software (Addinsoft, NY, USA) at a 95% confidence level (Fisher LSD, $p < 0.05$) to evaluate significant differences between means.

3. RESULTS AND DISCUSSION

3.1 Effect of simulated shipment on hydrolytic degradation

Free acidity is considered as an important parameter to determine the hydrolysis of triacylglycerol in olive oil. Moreover, acidity values are considered as a basic criterion to classify the different categories of olive oil. The results in Table 1 show that FA increased slightly during shipments to both destinations. In addition, there was a slight increasing trend in FA for EVOO LA shipped in a standard container compared with that before shipping, which was influenced by the increase in temperature during the simulated journey (PARADISO *et al.*, 2010). However, none of the shipped EVOO samples reached the limit of 0.8% accepted for the extra virgin olive oil category (EEC Reg. 1348/2013).

Table 1. FA, free acidity (g oleic acid 100 g⁻¹ oil); PV, peroxide values (meq O₂ kg⁻¹ oil); TBARS, thiobarbituric acid reactive substances value (mg of malonaldehyde equivalent kg⁻¹ oil); TP, total phenols (mg gallic acid kg⁻¹ oil) tested before simulation and after simulation of shipping in insulated and standard containers for evoo samples to the two final destinations (EVOO Q, Quebec and EVOO LA, Los Angeles).

Values (mean \pm standard deviation) with different superscript capital letters in each column and for each sample were significantly different between the simulated shipping conditions ($p < 0.05$; Fisher's test).

Sample	Experimental condition	FA (g oleic acid 100 g ⁻¹)	PV (meq O ₂ kg ⁻¹)	TBARS (mg of malonaldehyde equivalent kg ⁻¹)	TP (mg gallic acid kg ⁻¹)
EVOO Q	Before shipping	0.52 ^B \pm 0.04	11.7 ^C \pm 0.7	0.013 ^B \pm 0.001	353 ^B \pm 25
	Insulated container	0.59 ^A \pm 0.01	13.1 ^B \pm 0.3	0.012 ^B \pm 0.001	372 ^B \pm 38
	Standard container	0.60 ^A \pm 0.01	17.0 ^A \pm 0.8	0.016 ^A \pm 0.001	478 ^A \pm 30
EVOO LA	Before shipping	0.45 ^B \pm 0.01	8.8 ^C \pm 0.2	0.015 ^C \pm 0.001	259 ^A \pm 2
	Insulated container	0.45 ^B \pm 0.01	9.2 ^B \pm 0.1	0.028 ^B \pm 0.001	257 ^A \pm 8
	Standard container	0.48 ^A \pm 0.01	10.4 ^A \pm 0.1	0.040 ^A \pm 0.001	222 ^B \pm 3

Acid value results (Table 2) of GSO stored in the standard container for both simulated shipments were significantly higher in comparison with the thermally insulated samples and that before shipping. Considering the RO samples shipped to Quebec which, before starting the simulation, had an AV higher than the accepted limit of 0.6% for edible oils (CAC 2013a), the AV registered for the sample stored in the standard container was significantly higher than both the respective values for samples with and without thermal insulation.

Table 2. AV, acid values (mg KOH g⁻¹); PV, peroxide values (meq O₂ kg⁻¹ oil); TBARS, thiobarbituric acid reactive substance values (mg of malonaldehyde equivalent kg⁻¹ oil) of vegetable oil samples [grape seed oil (GSO) and rice oil (RO)] tested before and after simulation of shipping in insulated or standard containers to the two final destinations (coded as “Q” to Quebec and as “LA” to Los Angeles). Values (mean ± standard deviation) with different superscript capital letters in each column and for each sample were significantly different between the simulated shipping conditions (p < 0.05; Fisher’s test).

Sample	Experimental conditions	AV (mg KOH g ⁻¹)	PV (meq O ₂ kg ⁻¹)	TBARS (mg of malonaldehyde equivalent kg ⁻¹)
GSO Q	Before shipping	0.27 ^C ± 0.00	4.2 ^B ± 0.1	0.018 ^A ± 0.001
	Insulated container	0.36 ^B ± 0.03	6.3 ^A ± 0.9	0.020 ^A ± 0.003
	Standard container	0.43 ^A ± 0.00	6.2 ^A ± 0.1	0.017 ^A ± 0.002
RO Q	Before shipping	0.74 ^C ± 0.01	4.4 ^B ± 0.2	0.017 ^B ± 0.001
	Insulated container	0.86 ^B ± 0.03	4.8 ^A ± 0.1	0.016 ^B ± 0.002
	Standard container	0.98 ^A ± 0.08	4.1 ^B ± 0.1	0.022 ^A ± 0.003
GSO LA	Before shipping	0.24 ^B ± 0.04	1.6 ^B ± 0.0	0.018 ^C ± 0.001
	Insulated container	0.24 ^B ± 0.03	3.3 ^A ± 0.5	0.020 ^B ± 0.001
	Standard container	0.35 ^A ± 0.02	3.0 ^A ± 0.2	0.043 ^A ± 0.001
RO LA	Before shipping	0.46 ^A ± 0.01	3.3 ^B ± 0.3	0.014 ^B ± 0.001
	Insulated container	0.45 ^A ± 0.03	3.5 ^B ± 0.4	0.020 ^A ± 0.001
	Standard container	0.51 ^A ± 0.03	4.9 ^A ± 0.4	0.020 ^A ± 0.001

The results for the RO sample to Quebec revealed a drastic effect of temperature variation, and in particular for low quality edible oils. In fact, as recorded during the simulation in a standard container to Quebec, the temperature decreased to -10°C (Fig. 2). Such low temperatures probably facilitate hydrolytic processes due to water droplets in the liquid phase that surrounds the lipid crystals (KRISTOTT 2000). In the case of RO in the simulated shipment to Los Angeles, on the other hand, the change in AV after simulation in both the standard and thermally insulated containers was not significant; in this case, the samples experienced a slight temperature fluctuation during 13 days of simulated shipment before reaching the final destination.

3.2. Influence of simulated shipment on oxidation stability

In order to estimate the effect of shipment on EVOO and other vegetable oils, oxidation quality was tracked by evaluating i) PVs, which indicate the increase in primary oxidation products, such as hydroperoxides, and ii) TBAR values, which detect the formation of malondialdehyde from fatty chains with three or more double bonds (FRANKEL, 1991), and indicate the trend in secondary oxidation products in edible oil. As seen in Table 1, the PV was significantly higher in the EVOO sample for which the simulated shipment was conducted in a standard container compared to that shipped in a thermally insulated container for both destinations. TBARS values were also significantly higher when a standard container was used to transport EVOO samples compared with those subjected to simulation in a thermally insulated container for both destinations. These results suggest that thermally insulated containers have a beneficial effect, compared with a standard container, in terms of protecting EVOO samples against oxidative stress. Moreover, starting from similar values for both samples before shipping, higher TBARS

values were reported for EVOO sent to Los Angeles compared with the sample sent to Quebec; this may be related to the higher temperature stress applied in the Los Angeles simulation (Figs. 2 and 3).

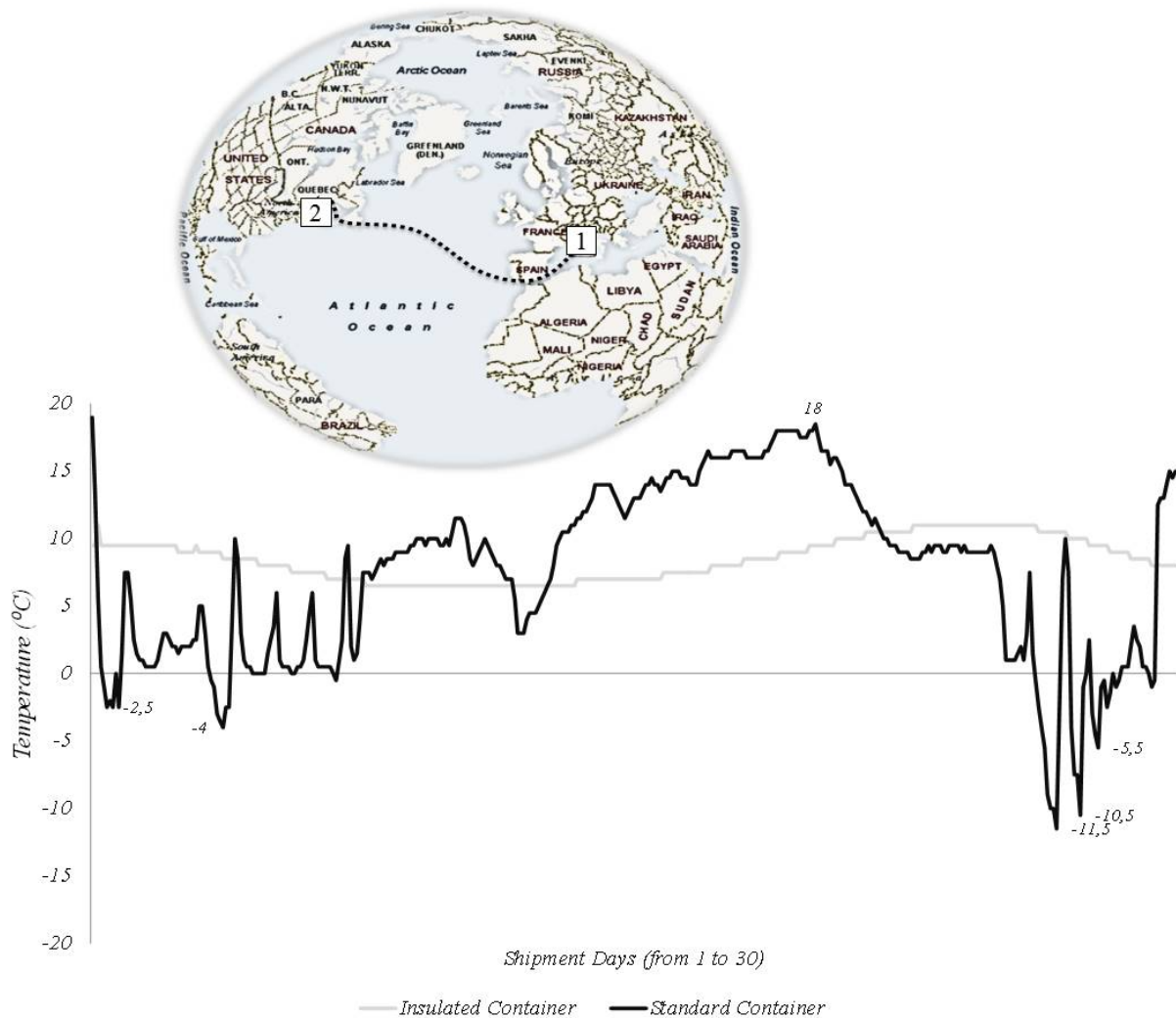


Figure 2. Temperature profile monitored using data loggers for the Quebec simulation (in the world map, 1: Livorno port; 2: Quebec port). a: inside standard container; duration: 30 days; highest temperature: 19°C; lowest temperature: -11.5°C. b: inside thermal insulated container; duration: 30 days; highest temperature: 11°C; lowest temperature: 6.5°C.

Regarding the other vegetable oils, the PVs (Table 2) had higher values after simulation compared with those before shipping, for both destinations, except for RO shipped in a standard container to Quebec. Considering RO to Los Angeles, a higher increase was observed in PVs in a standard container compared with thermally insulated samples, which indicate more advanced formation of peroxides in the standard container. On the other hand, the lower PV values seen in RO to Quebec in a standard container compared with samples shipped in an insulated container reveals possible additional transformation of peroxides to secondary oxidation products, which was also confirmed by the increase in TBAR observed in the same sample (Table 2). The higher impact on oxidative status on all

edible oils by the Los Angeles simulation is also demonstrated by considering the changes in total phenols in EVOO (Table 1): these minor components, in addition to their nutritional role, act as antioxidants in EVOO (BENDINI *et al.*, 2007). Before simulation, EVOO samples contained about 353 and 259 mg gallic acid kg⁻¹ oil, respectively, for samples sent to Quebec and Los Angeles (Table 1); after shipping, these values tended to decrease in standard container for the samples sent to Los Angeles. This reduction was more pronounced for samples stored in the standard container than after the non-thermally insulated journey due to the effect of higher temperature stress (Fig. 3). The anomalous increase in total phenolic content registered for the EVOO shipped to Quebec after simulation in standard container could be attributed to the higher extractability of phenolic molecules after a crystallization and subsequent thawing out caused by the low temperatures reached during the simulation (Fig. 2). On the other hand, for the sample EVOO LA shipped at higher temperature, this effect was not observed.

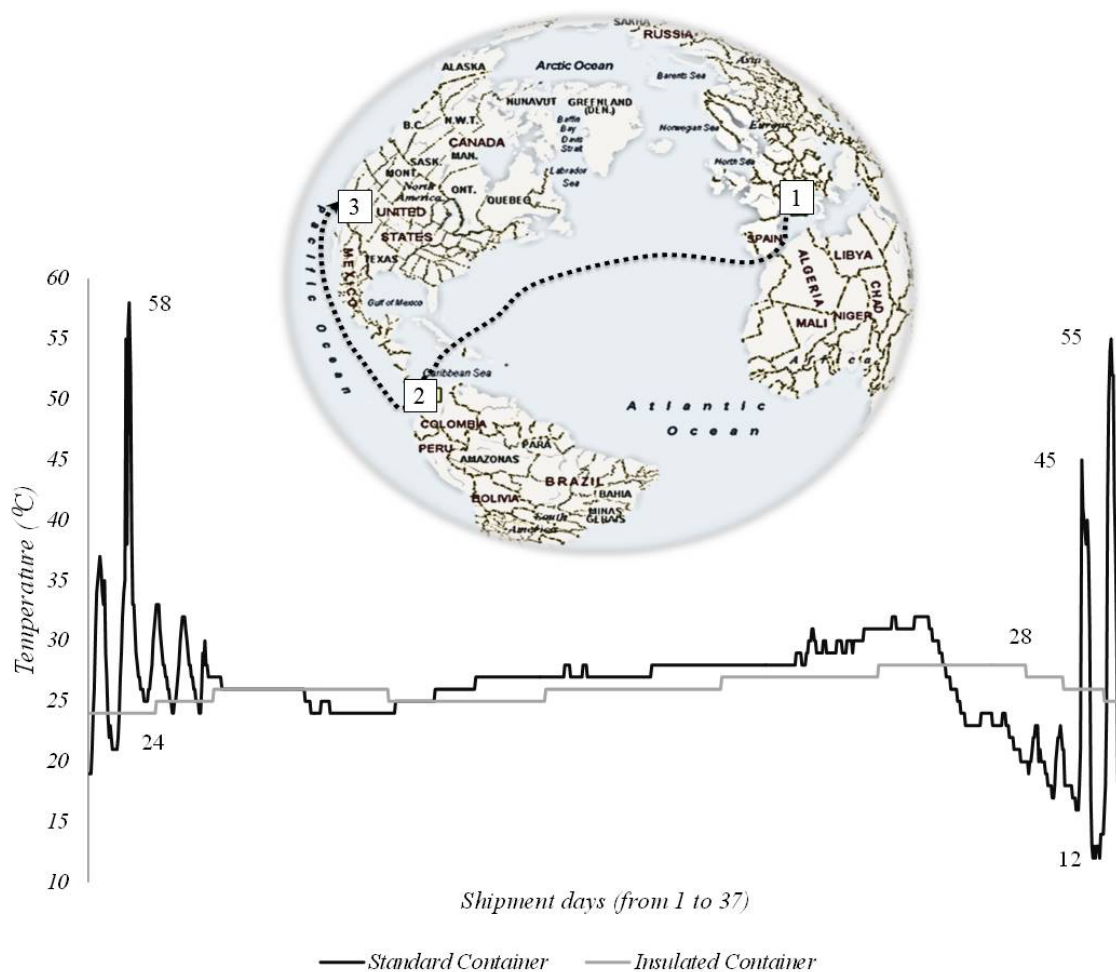


Figure 3. Temperature profile monitored using data loggers for the Los Angeles simulation (in the world map: 1, Genoa port; 2, panama canal ; 3, Los Angeles port). duration: 37 days, highest temperature: 58°C, lowest temperature: 11.5°C.

3.3. Influence of simulated shipment on physical and sensory properties

Color changes in EVOO reflect the visual color appearance that is considered to be an important factor in consumer satisfaction (MOYANO *et al.*, 2010). The color of olive oils, in general, is principally affected by two classes of minor compounds, namely chlorophylls and carotenoids. The degradation of these compounds is due to different conditions of stress, such as temperature and light, which may alter color in addition to clarity and transmittance (SIKORSKA, *et al.*, 2007). Color indexes were expressed as chromatic coordinates: L* corresponds to brightness and positive b* to yellowish color, while negative a* corresponds to light green color (MINGUEZ-MOSQUERA *et al.*, 1991). As seen in Table 3, there were significant changes in the brightness (L*) and b* indices for EVOO samples sent to Quebec after simulation in the standard and insulated containers (more bright and more yellowish). However, a reduction in L* values (meaning less bright oils) was seen in both shipping conditions for the simulated shipment to Los Angeles. A reduction was also observed for b* values (less yellow toward light blue) of samples shipped to Los Angeles, corresponding to the degradation of yellow chromophores (pigments), that function as natural antioxidants, such as carotenoids and pheophytins (PSOMIADOU and TSIMIDOU 2002), since oxidation is promoted by the increased temperature (MORELLO *et al.*, 2004) during the simulation to Los Angeles (Fig. 3). As previously reported, degradation of natural pigments such as carotenoids occurs at around 40°C (THAKKAR *et al.*, 2009). Moreover, an increase in a* values (partial loss of green color toward redness) was recorded for samples sent to Los Angeles: such a partial loss of green color, in general, may correspond to partial degradation of chlorophylls, which are partially converted into other gray/brown compounds, and specifically to pyropheophytin a which is formed from pheophytin a due to degradation triggered by inadequate temperatures during the storage of oil (APARICIO-RUIZ *et al.*, 2014). Consequently, the increased degradation of chlorophyll and carotenoid pigments is likely related to the increased temperature (up to 58°C) in the final stages of the Los Angeles simulation (Fig. 3).

In addition, variations in water amount and turbidity were not significant (Table 3) in either simulation. Sensory analysis, realized according to the EU Reg. 640/2008, is an essential technique for the assessment of the quality of EVOO. The sensory evaluation (results not shown) indicated that no sensory defects developed after simulated shipment to Quebec or Los Angeles, and all samples remained within the “extra virgin” category in both thermally insulated and standard containers.

Table 3. Color coordinates (L*, a*, b*); TD, turbidity (NTU); WA, water amount (mg kg⁻¹ oil) before and after simulated shipping in an insulated and standard container for EVOO samples to the two final destinations (EVOO Q, Quebec and EVOO LA, Los Angeles).

Values (mean ± standard deviation) with different superscript capital letters in each column and for each sample were significantly different between the simulated shipping conditions (p < 0.05; Fisher’s test).

Samples	Experimental conditions	L*	a*	b*	TD (NTU)	WA (mg kg ⁻¹ oil)
EVOO Q	Before shipping	54 ^B ± 0.1	4.9 ^A ± 0.0	80 ^B ± 0	11.7 ^A ± 0.2	719 ^A ± 98
	Insulated container	55 ^A ± 0.1	4.8 ^B ± 0.0	84 ^A ± 0	11.3 ^A ± 0.1	621 ^A ± 6
	Standard container	55 ^A ± 0.1	4.6 ^C ± 0.0	84 ^A ± 0	11.5 ^A ± 0.2	708 ^A ± 92
EVOO LA	Before shipping	63 ^A ± 0.0	4.3 ^B ± 0.1	89 ^A ± 0	11.6 ^A ± 0.2	650 ^A ± 30
	Insulated container	50 ^B ± 1.4	5.8 ^A ± 0.2	71 ^C ± 1	11.5 ^A ± 0.2	607 ^A ± 64
	Standard container	52 ^B ± 1.5	5.5 ^A ± 0.2	79 ^B ± 2	11.4 ^A ± 0.1	562 ^A ± 72

4. CONCLUSIONS

It is important to point out that this study is related to two specific simulations, and thus the results cannot be generalized to all shipments of vegetable oils to Los Angeles or Quebec. From parallel study of two simulated shipments to different destinations with different thermal conditions, it was found that thermal isolation is associated with significant benefits in terms of avoiding an increase in degradative reactions for edible oils, and especially on oxidative status. Considering the different parameters evaluated, the quality of the edible oils subjected to the simulation to Quebec was higher than those shipped to Los Angeles, which was due to the different thermal profiles of the two journeys. The aim of future studies is the adoption of a proposed ex-post simulation analysis on edible oils having different ages, shipped in different periods of the year and to different destinations, in agreement with specific logistic decisions (storage, material handling, transportation modes, etc.) and packaging solutions including primary, secondary, tertiary packaging, and containment equipment.

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