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Valorization of tomato peel waste carotenoids in different oil matrices

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ABSTRACT/RESUME

Abstract : *The aim of this study is to enrich different oil matrices with carotenoids and lycopene from tomato peel waste.*
The present study tested two hypotheses: 1) fatty acid chain length and degree of unsaturation affect diffusivity of lycopene and β-carotene in fat matrix; and 2) diffusivity of carotenoids in fat changes with the quantity of carotenoids extracted.
To evaluate the effect of fatty acid profile on carotenoid diffusivity, dried tomato peel (DTP) was extracted in ten different mixtures of sunflower oil (SO), goat's butter (GB) and palm stearin (PS).Proportions of each fat type in the mixture was calculated using a mathematical model. Three concentrations of DTP were tested, 2.22, 4.44 and 6.66%.
Dry tomato peel used in this study was rich in carotenoids, lycopene especially (188.2 mg/100 mL). Our results confirm that fatty acid profile of the fat matrix affects diffusivity of carotenoids. We concluded that at lower concentrations of DTP, lycopene has higher diffusivity with short chain fatty acid, while with higher concentrations of DTP, diffusivity of lycopene is favored with longer chain fatty acids. Diffusivity of β-carotene is not associated directly with the fatty acid chain length. Its pattern might be due to other factors of competition and molecular structure.
There was no evidence that the unsaturation degree of fatty acids affects carotenoid's diffusivity in fat.

I. Introduction

Health and Human (HHealth) benefit associated with high intake of fruits and vegetables is widely recognized by health professionals and food scientists alike. Dietary guidelines including the food pyramids and my plate [1] or the latest version of the food pyramid recommended by the Flemish Institute for Healthy Living [2] agreed all on recommending increased consumption of plant-based foods for the prevention of chronic diseases and maintaining good health. Since the interest in fruits and vegetables has increased, interest in phytochemicals or the biologically active compounds has grown [3].

Food scientists have created novel foods that were adapted to the customer demand for "healthy foods". However the definition of healthy food is evolving as we know more about our nutrition and health. For example, the Food and drug Administration (FDA) is currently discussing changing the requirement for labeling "healthy food" a food that was in the 1990th low in fat, regardless of the nature of fat, to a food that contains a "meaningful amount" of foods that comprise a healthy diet and that does not contain any calorie sweeteners or synthetic colors [4]. Nowadays, food industry has tendency to replace additives that are synthetic molecules by plant extracts or by-products of the agro-food industry

rich in phytochemicals with particular interest toward antioxidants and more especially, carotenoids. Carotenoids are tetrapenes, fat soluble molecules that can contain oxygen (xanthophylls) or can be constituted by pure chain of carbon and hydrogen (carotenes). They are almost exclusively produced in plants which constitute the main source for human and animal intake, in addition to algae. In the human diet, carotenoids of interest are vitamin A precursors mainly β-carotene. Lutein and zeaxanthin are believed to have a protective activity against age-related macular degeneration, although results from *in vitro*, *in vivo* and epidemiological studies are inconclusive [5]. The role of other carotenoids such as lycopene in the human and animal organisms are still not very known, but importance related to their anti-carcinogenic and anti-atherogenic activities is still explored [6].

Tomato and tomato products have been promoted for their health benefit associated with their high content in carotenoids, mainly β-carotene and lycopene. However, it has been shown that bioavailability of carotenoids from fruits and vegetables is lower than it has been anticipated [7]. Factors that affect bioavailability of carotenoids include: species; molecular linkage; amount; matrix; effectors; nutrient status; genetics; host-related factors; and interactions among these variables [8]. Their activity at the molecular level depends additionally on their structural and geometrical configuration [9].

Our present study focuses on the effect of the type of fat on the diffusivity of carotenoids from tomato peel, very rich in lycopene and β-carotene. Few studies have tested the effect of type of fat, i.e. its fatty acid profile on the diffusivity and the bioavailability of carotenoids. In a previous study carried out by our research group on the valorization of low quality edible oil with tomato products, i.e. peel and puree, we found no significant difference in carotenoid solubility in refined olive oil, extra virgin olive oil and refined sunflower oil. We hypothesized that the no difference was due to the similarity in fatty acid composition of the studied oil matrices [10]. According to Borel et al. (1996), solubility of apolar (β-carotene) and polar (zeaxanthin) carotenoids increase with decreased fatty acid chain length, while unsaturation degree might not have an effect in carotenoid's solubility [11]. In an effort to bring more insight into the effect of fat type in carotenoids diffusivity, we carried out this work with the objectives 1) to assess the effect of the fatty acid profile of three different fats (sunflower oil, goat's butter and palm oil) on the diffusivity of carotenoids from dry tomato peel, and 2) to assess the effect of the amount of dry tomato peel mixed in the three fat types on its diffusivity.

II. Materials and methods

II.1. Materials

Fresh tomatoes were purchased from the local market in February 2017. After opening the packet, the product was stored at -20°C until preparation. The peel of the tomatoes was separated from the flesh using a sharp knife and was frozen immediately (the flesh was discarded). The sample was then freeze-dried, dried tomato peel (DTP), placed in oxygen barrier bags and stored frozen at -20°C until analysis.

II.2. Fat choice

The three fat types were selected for the difference in their fatty acid composition in length and saturation. We selected refined sunflower oil (SO), goat's butter (GB) and palm stearin (PS). Goat's butter is a solid animal fat, it was bought from the region of Biskra (Eastern Algeria) in March 2017 and was stored in the freezer at -15˚C until its use in the different tests. Refined sunflower oil is a fluid oil that was bought from a local shop in 2L bottles and was stored at room temperature. Palm stearin was selected for its similarly long chain fatty acids but saturated (compared to sunflower oil). Palm stearin is a solid fraction of the palm oil and was provided by the company SOFAMAR (Algeria).

II.3. Dried tomato peel quantity

Three concentrations of DTP were selected: 2.22, 4.44 and 6.66% (Table 1). These concentrations were based on a theoretical content of 1.5 mg lycopene / g DTP to obtain final theoretical concentrations of 3.33; 6.66 and 9.99 mg lycopene /100 g fat [12-14].

II.4. Sample preparation

Totest the diffusivity of DTP carotenoids in different fatty acid profiles, a statistical model was used to optimize the proportions of the three types of oils used. The software Minitab (Minitab 7.0) basis its calculation on a cubical comparison of the second degree including the three types of fat tested: refined sunflower oil (SO), goat's butter (GB) and palm stearin (PS). Ten mixtures have been defined by this exercise which are summarized in table 1.

Preparation of the different mixtures containing PS needed to be performed at high temperature because of its solid consistency at room temperature (melting point between 51 and 55˚C). For this, all these mixtures were prepared (including weighing, steering, saponification and decantation) using an oven and a water bath set at 60°C. Another technical challenge that we faced was the formation of a stable aqueous phase-in-oil emulsion. To break the emulsion and to allow the

separation of the two phases, ethanol and hot water were added.

The mixtures of fat and different concentrations of DTP were heated if needed (depending on the PS content), steered using a vortex and then incubated in an incubator (HEIDOLPH Unimax 1010) with continuous agitation at 60˚C for three days. Under these conditions, factors that might limit the diffusion of the molecules into the fat matrix with high fusion point and viscosity (those containing high amounts of PS) were limited.

Thirty tubes (capacity of 50 mL) were prepared in total (ten mixtures of fats and three concentrations of DTP). Each tube contains 45 g of oil/fat mixture, where one of the concentrations of DTP was added (Table 1). Fat samples thus obtained were homogenized with a cyclone I.Q.^{2}Virtis blender for 3 days, followed by centrifugation at 6240 g for 15 min on a HERAEUS Megafuge 1.0 R centrifuge (Germany). The oil phase was recovered and stored at 4 °C until analysis.

Table 1.*Fat mixtures enriched with dry tomato peel (2.22, 4.44 and 6.66 % DTP).*

Mixture	SO	GB	PS
1	1.00000	0.00000	0.00000
2	0.50000	0.00000	0.50000
3	0.00000	1.00000	0.00000
4	0.33333	0.33333	0.33333
5	0.00000	0.50000	0.50000
6	0.50000	0.50000	0.00000
7	0.16667	0.16667	0.66667
8	0.16667	0.66667	0.16667
9	0.66667	0.16667	0.16667
10	0.00000	0.00000	1.00000

SO: Sunflower oil
GB:Goat's butter
PS:Palm stearin

II.5. Fatty acid profile of the fat and oils

In a 5 mL-tube with screw cap, 0.1 g of the tested fat (SO, GB or SP) were weighed. 2 mL of heptane were added and the mixture was agitated. Later, 0.2 mL of methanolic potassium hydroxide solution (2N) were added, then the tube was shacked vigorously for 30 seconds, and then allowed to settle. The organic phase that contains the methyl esters was recovered, stored in a refrigerator before being injected in the CG-MS (Chromatograph : Hewlett Packard Agilent 6890 plus Mass Spectrometer: Hewlett Packard Agilent 5973) for fatty acid profile analysis [15]. For this analysis, the following conditions were used: a sample of 0.2 µL was injected in a splitless mode at 250 °C. The system includes a column (HP-5MS, 30 m Length x 0.25 mm Internal diameter with 0.25 µm film thickness). The stationary phase was composed of 5% phenyl and 95% dimethylpolysiloxane. The carrier gaz was helium (purity N6), with a flow rate of 1.5 mL/min. The thermal program was as follow: 70 °C maintained for 5 min, followed by an increase in a rate of 5 °C/min until the system reaches 130 °C. Then again the temperature was maintained for 2 min followed by an increase of 3 °C/min until it reaches 220 °C. This temperature was kept for 7 min; then a third increase with 6 °C/min until a temperature of 240 °C was reached and maintained for 5 min. the whole cycle lasted 64 min for each sample.

The mass detector uses a Scan TIC (30 – 550) as a mode of analysis; solvent period (8 min); interface temperature (270 °C); electron ionization as the source of ions; temperature at the source of 230 °C; the electrons are accelerated to 70 eV in the region between the filament and the entrance to the ion source block, and then analyzed by an analyzer, quadrupole type.

II.6. Carotenoid analysis

Carotenoids were extracted according to Calvo (2008) [16]. One gram of dry tomato peel or 10 g of fat enriched with carotenoids were mixed with 60 ml of 10% KOH-methanol for 10 s using an Ultraturrax macerator (Janke&Kaudel, IKA). The mixture was stirred using a magnetic stirrer under darkness at 40 °C for 2 h. After saponification, 60 mL of distilled water, 30 mL of hexane and 3 mL of methanol were added and the mixture was shaken vigorously; hexane containing the lycopene and other lipid-soluble components was collected and stored at 4 °C in the dark. The water phase was mixed again with 30 mL hexane and 3 mL methanol with shaking. Hexane was removed as before and the water phase was mixed again with 30 mL of hexane and 3 mL methanol. The extraction of lycopene from the aqueous phase was performed until hexane was colorless.

All hexane phases obtained were washed three times by adding 200 mL distilled water to eliminate alkali residues. Then hexane containing the lycopene was filtered through a 1PS phase separator filter (Whatman) to eliminate residual water and immediately evaporated under vacuum (<30°C), redissolved in 2 mL of hexane and stored at -20 °C prior to analysis. All extractions were performed in the dark.

The carotenoid analysis was carried out according to Soto-Zamoraa et al. [17] and Navarro et al. [18].

The analyses were carried out in triplicate and the concentrations were expressed as mg/100 mL of extract, as follows:

Lycopene (mg/100 ml extract) = - 0.0458(Abs $_{663}$) + 0.204 (Abs$_{645}$) + 0.372 (Abs$_{505}$) - 0.0806 (Abs$_{453}$).

β-carotene (mg/100 ml extract) = 0.216 (Abs$_{663}$) – 1.22 (Abs$_{645}$) – 0.304 (Abs$_{505}$) + 0.452 (Abs$_{453}$)

II.7. Statistical analysis

Data were analyzed using the analysis of variance ($p < 0.05$) to estimate the differences among values of compounds tested. Results were processed by the one-way analysis of ANOVA-MANOVA; the post-hoc test was performed (Duncan's test was applied) to indicate significant differences within individual groups.

III. Results and discussion

III.1. Tomato peel lycopene content

Contents in lycopene and other carotenoids vary mostly with growth conditions (temperature, light...). It has been found that tomatoes growing outdoors in the field contain higher concentrations of lycopene ranging between 5.2 and 23.6 mg/ 100 g; while tomatoes growing under cover (greenhouse) contain concentrations of lycopene ranging between 0.1 and 10.8 mg / 100 g [19]. Cultivar and ripening stage of tomatoes might affect the content in lycopene and in other phytochemicals [20]. Our results show that DPT contains 188.2 mg lycopene /100 g; a concentration that is higher than that found by Benakmoum et al. [10] in similar material (43.6 mg lycopene / 100 g DTP) and close to the one we found in a previous work with 126.2 mg lycopene / 100 g DTP [21]. Differences in concentrations of lycopene are due to differences in cultivar, season of harvest, in addition to between-year differences in climatic conditions. However all the results show high contents of tomato by-product in lycopene and ther phytochemicals emphasizing the importance of its valorization.

III.2. Fatty acid profile of fat

Fatty acid profile shows that SO contains mainly seven fatty acids, five of which are saturated (Table 2). The analysis confirms the high proportion of long chain unsaturated fatty acids at 86.26%; 55.31 % linoleic acid and 30.95 % oleic acid. In contrast, GB is particularly rich in short chain saturated fatty acids compared to the other two tested fats. It has also the particularity of containing fatty acids with odd number of carbon atoms, an important characteristic of animal-origin fat. GB contains capric acid (C10:0; 7.43%), lauric acid (C12:0; 3.38%) and myristic acid (C14:0; 7.23%). Palm stearin is rich in saturated long chain fatty acids represented mainly by palmitic acid (60% of total fatty acids).

Table 2.*Fatty acid profile of sunflower oil, goat's butter and palm stearin.*

Fatty acid	SO	GB	PS
C8 : 0		2.39	
C10: 0		7.43	
C12: 0		3.38	0.11
C14 : 0		7.23	1.45
C15 : 0		0.79	
C16 : 0	8.01	20.18	60.70
C17 : 0		0.66	
C18 :2	55.31	18.63	5.44
C18 :1	30.95	22.14	26.24
C18 :0	4.13	10.47	5.42
C20:0	0.24	0.19	0.35
C22:0	0.64	0.14	
C24 :0	0.17		
∑ saturated fatty acids %	**13.19**	**52.86**	**68.03**
∑ unsturated fatty acids %	**86.26**	**40.77**	**31.68**

SO: Sunflower oil
GB:Goat's butter
PS:Palm stearin

III.3.Optimization of models of diffusivity of DTP carotenoids

To determine whether the selected models were appropriate and whether the regression hypotheses were satisfied, we plotted the residual values' graphs, contour plots, and optimization diagram, separately for β-carotene and lycopene and for each DTP concentration used, separately.

III.3.1. Residual values' graphs. [lycopene a to d; β-carotene e to h]

Fatty acidAt 2.22 % DTP, the straight line in figure 1a shows that the values were normally distributed and that the mixtures had positive effect on lycopene diffusivity. When adjusted values were plotted against residual values (figure 1b), the plot shows an important variation in observed data. This is explained by the difference in the fatty acid composition of the tested fat mixtures. The histogram of residual values (figure 1c) showed the symmetric pattern of the values, indicating no outliers. The slow modification of the residual

values' signs on the plot against order function indicate a negative correlation among the residual values (figure 1d), which might affect the results of the adjustment model. The same patterns were found for β-carotene (figure 2 e, f, g and h). The plots showed a normal distribution of values but with a high variation among the observed data. There was also a negative correlation among the residual values.

At 4.44% DTP, lycopene concentrations followed also a normal distribution (figure 3a), but with a high variation in observed data (figure 3b) that might have resulted from the higher concentration of DTP and consequently the higher diffusivity of lycopene. Histogram of residuals showed this time an asymmetric pattern between observed and adjusted values (figure 3c), which means that this set contains outliers. The fast change in residuals' sign (figure 3d) shows that there was a positive correlation between residuals. At the same concentration of DTP, residuals plots concerning β-carotene (figure 4 e, f, g, h) show a proximity of values but with a high variability among residuals. Histogram of residuals shows a symmetric pattern between residuals, and the fast change in residuals' sign demonstrates a positive correlation (same like lycopene).Figures5 and 6 represent the diagram of residual values for lycopene and β-carotene for mixtures containing 6.66% of DTP. Figure 5 (a, b, c and d) shows that there was a high variability and symmetric pattern of residuals, indicating again an increase in the diffusivity of lycopene at this concentration of DTP. While for β-carotene, figure 6 (e, f, g, h) shows a homogenous distribution of values, with a low variability in residuals. This could be explained by a saturation state that started to be reached at this concentration. As for lycopene, there was a positive correlation between residuals.

III.3.2. Contour plots

When DTP was added at 2.22 %, contour levels indicate a pic at approximately 50% SO and 50% GB for both tested carotenes. Respective concentrations in that zone are 1.4 and 1.6 mg / 100 mL for lycopene and β-carotene (figure 7 a and b). At 4.44 % DTP, contour levels show a pic at a mixture of PS and GB (figure 8a) for lycopene. Lycopene concentration in this zone was higher than 2.8 mg/ 100 mL, which indicates an increase of 100% compared to the enrichment obtained with 2.22 DTP. For β-carotene, contour levels show a pic at 100% GB (figure 8b), resulting in a concentration of 2.7 mg β-carotene / 100 mL, equivalent to an increase of 40% compared to a concentration of 2.22 DTP (in a mixture 50% SO and 50% GB).

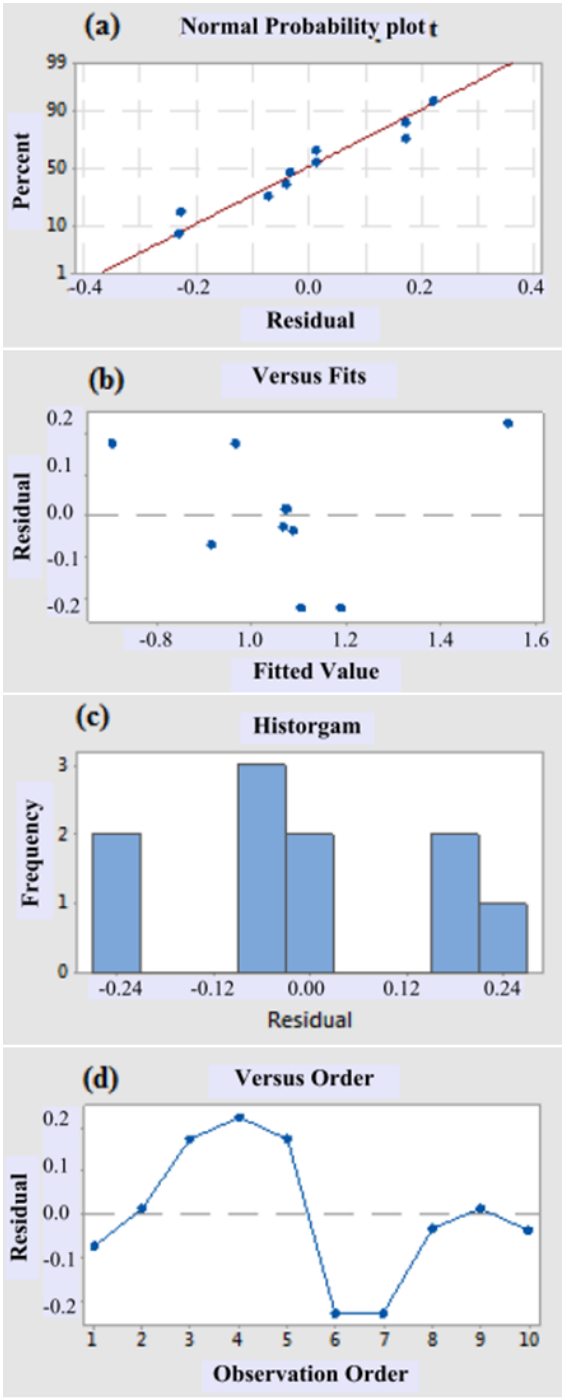

Figure 1 . *Residual value graphs of lycopene (a,b,c,d) in fat mixture with 2.22% DTP.*

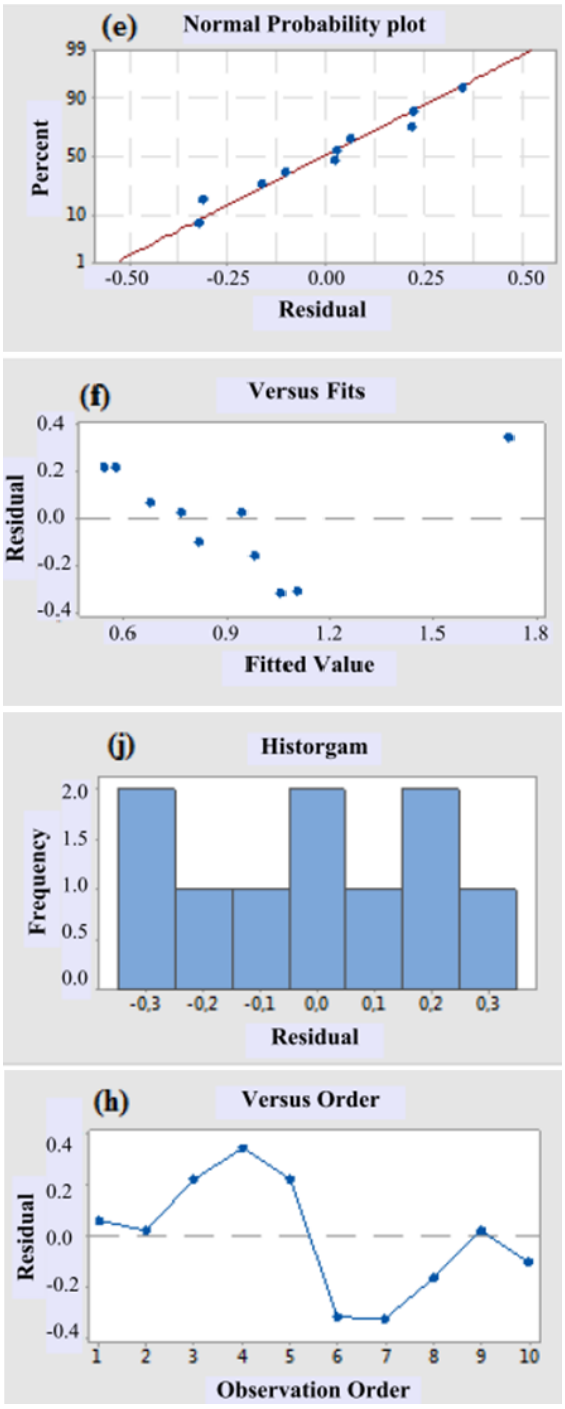

Figure 2. *Residual value graphs of carotene (e,f,g,h) in fat mixture with 2.22% DTP.*

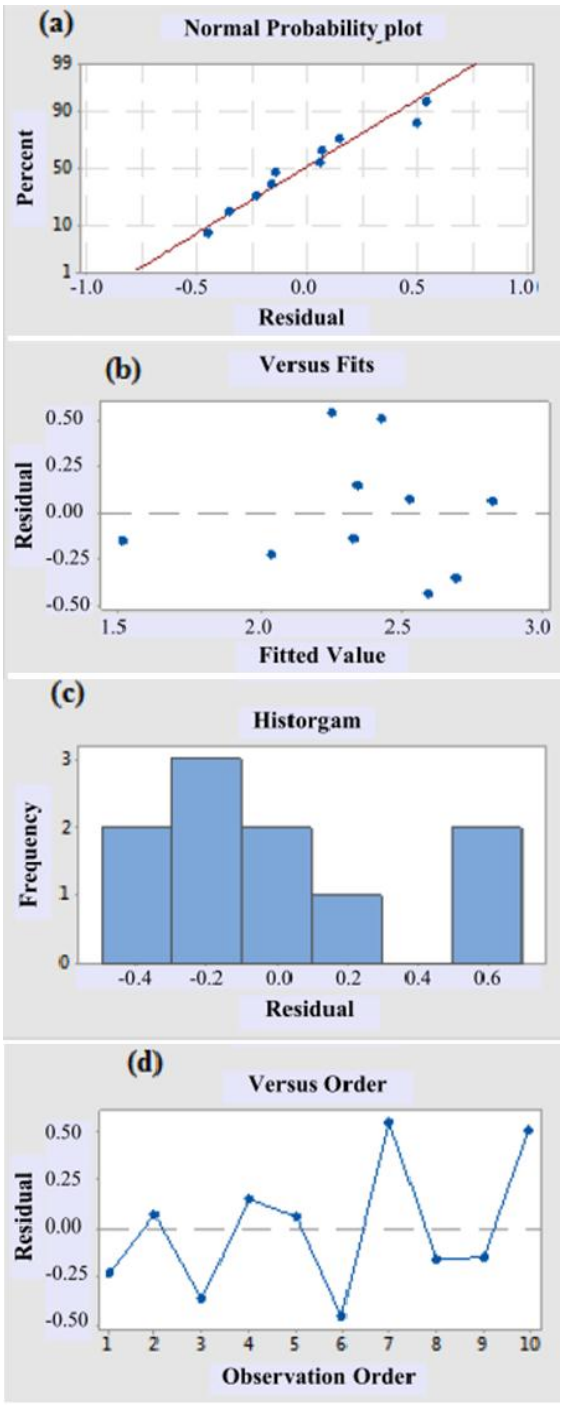

Figure 3. *Residual value graphs of lycopene (a,b,c,d) in fat mixture with 4.44% DTP.*

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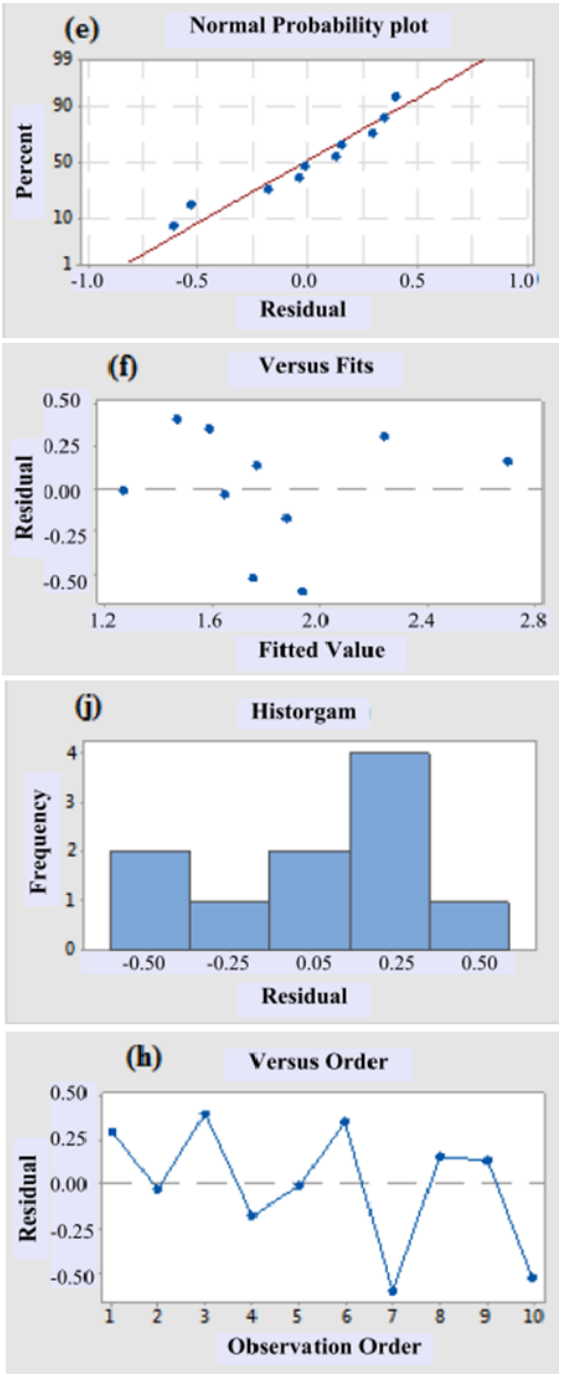

Figure 4. *Residual value graphs of carotene (e,f,g,h) in fat mixture with 4.44% DTP.*

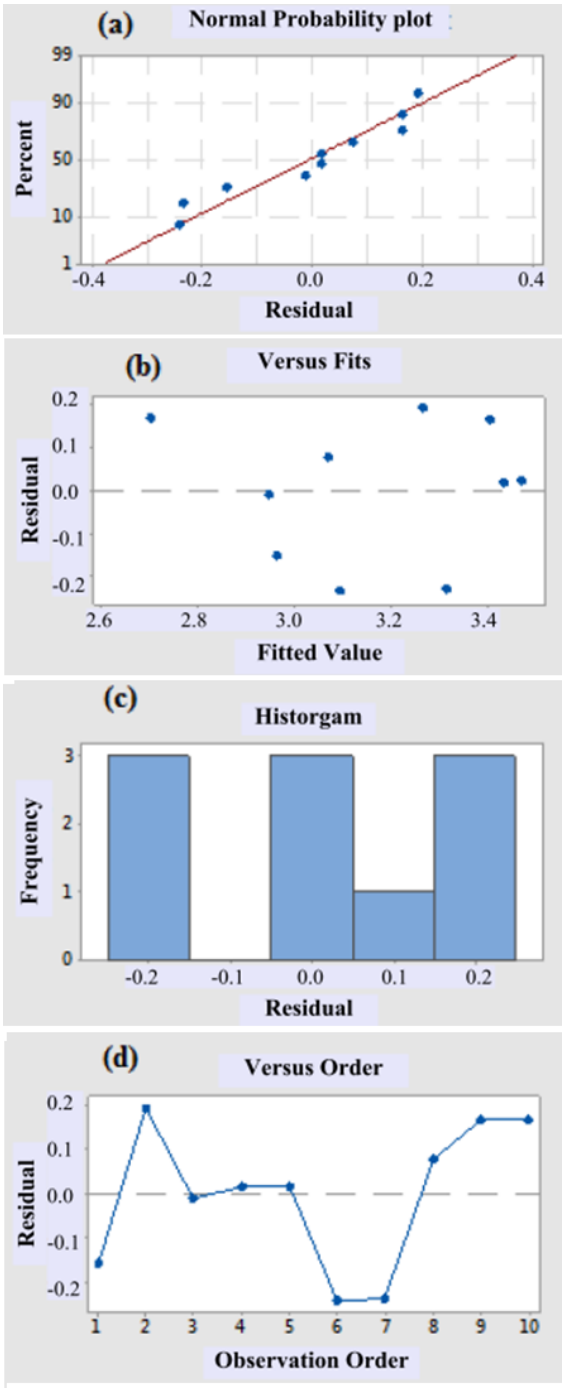

Figure 5. *Residual value graphs of carotene (a,b,c,d) in fat mixture with 6.66% DTP.*

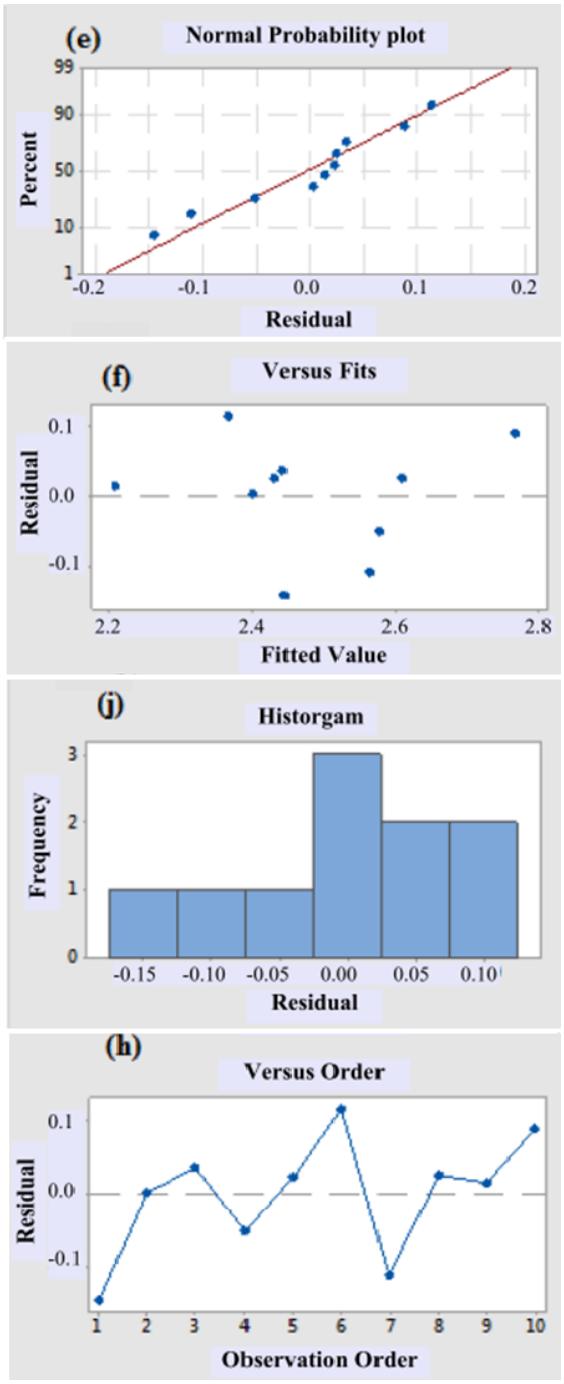

Figure 6. *Residual value graphs of carotene (e,f,g,h) in fat mixture with 6.66% DTP.*

When DTP was added at 6.66%, maximum diffusivity of lycopene was obtained with a mixture containing 100% SO (figure 9a), resulting in a concentration of 3.4 mg/ 100 mL of lycopene. While highest diffusivity of β-carotene was obtained in 75% SO and 25% PS, resulting in a concentration of 2.7 mg of β-carotene/ 100 mL.

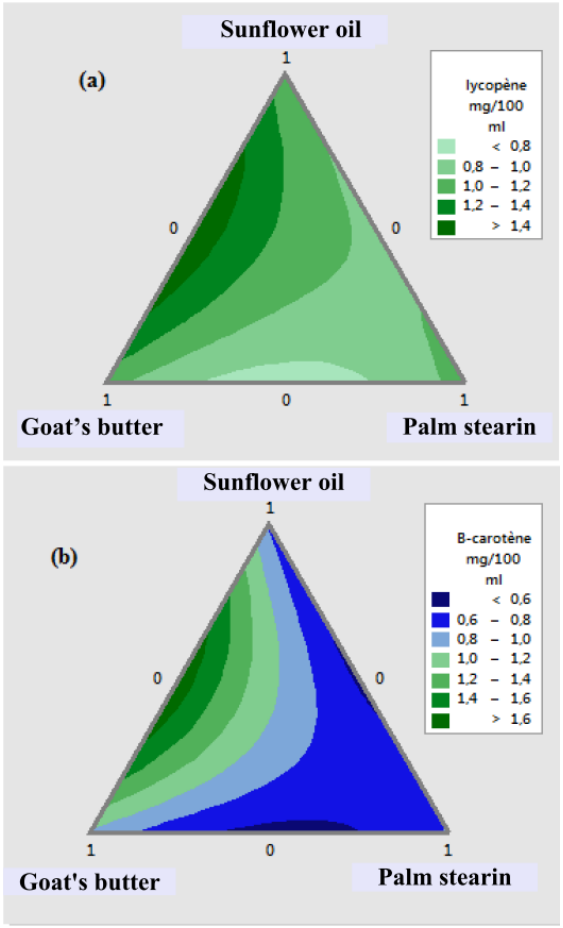

Figure 7. *Contour diagram of lycopene (a) and β-carotene (b) in fat mixture with 2.22% DTP.*

Overall, these results show that lycopene diffusivity increases with added DTP amounts, while β-carotene reached a saturation at 4.44% DTP. Diffusivity of both carotenoids at lower concentrations was better in a mixture of SO and GB. At higher concentrations, carotenes diffused better in SO.

III.3.3. Optimization diagrams

To assess the effect of the fatty acid profile of the different fats used in this study on the diffusivity of DTP carotenoids, we have plotted the optimization diagram of response. The relationship between the predicted responses of the three dependent variables (SO, GB and PS) and the desirability of the responses is called desirability function.

This analysis allows us to determine visually the proportions of the predictive variables that result in the optimal responses of the dependent variables.

Optimal mixture for a concentration of 2.22 % added DTP is constituted of 2.86% SO, 97.14% GB and 0% PS for lycopene; and 33.33% SO, 32.62% GB and 34.04% PS for β-carotene (figure 10). With an individual desirability of 1.0 for lycopene and β-carotene, the predicted response was 1.13 mg /100 mL and 0.967 mg/100 mL for lycopene and β-carotene, respectively. With 4.44% added DTP

(figure 11), the optimal mixture contains 33.33% SO, 57.96% GB and 8.71% PS for lycopene, and 23.25% SO, 59.60% GB and 17.15% PS for β-carotene. With an individual desirability of 1.00 for lycopene, and 0.99 for β-carotene, the expected response of carotene's diffusivity is 2.14 mg lycopene / 100 mL and 1.90 mg β-carotene /100 mL.

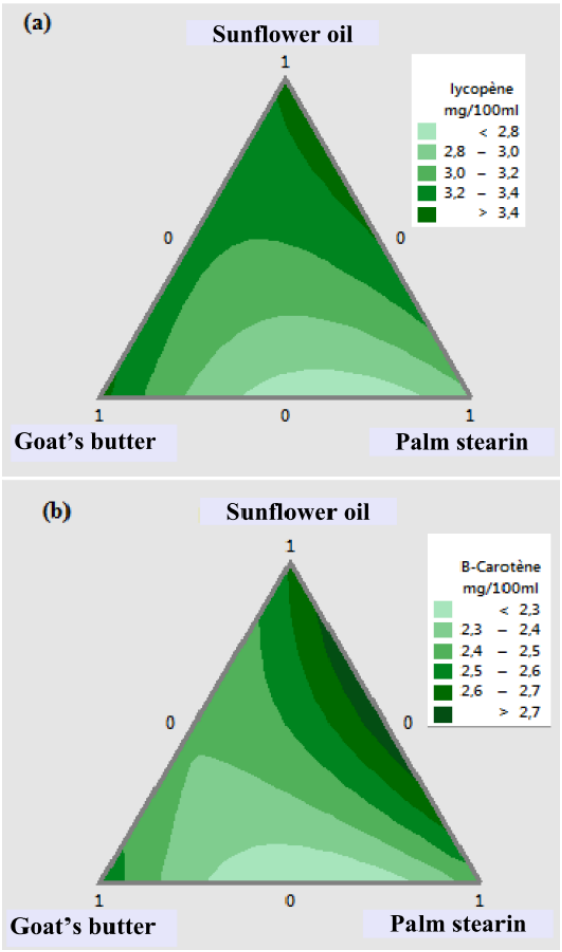

Figure 8. *Contour diagram of lycopene (a) and β-carotene (b) in fat mixture with 4.44% DTP.*

At 6.66% added DTP, optimal mixtures were respectively for lycopene and β-carotene of 16.77% SO, 0% GB and 83.23% PS; and 88.65% SO, 11.35% GB and 0% PS (figure 12). With an individual desirability of 1.00 for both carotenes, the expected response of diffusivity was 3.15 mg/100 mL for lycopene and 2.53 mg/100 mL for β-carotene.

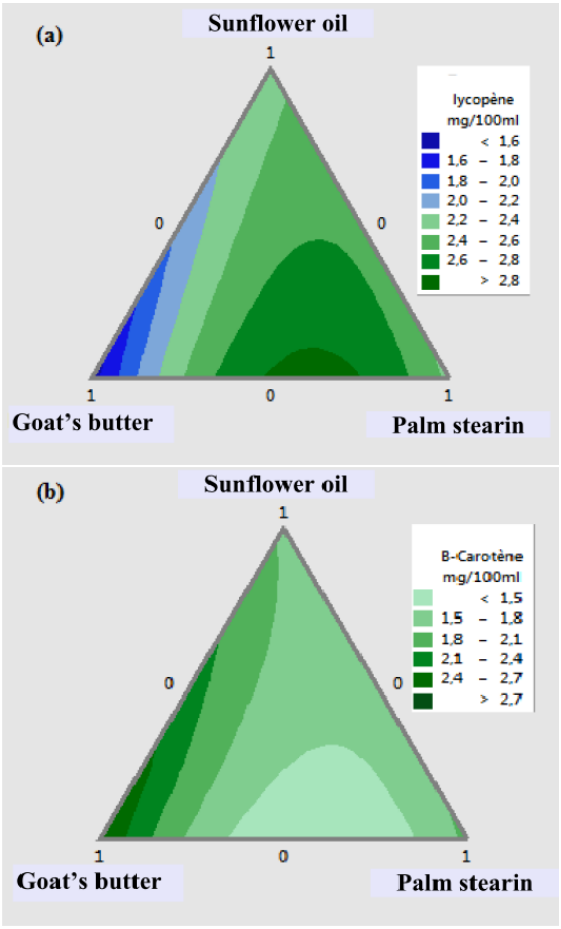

Figure 9. *Contour diagram of lycopene (a) and β-carotene (b) in fat mixture with 6.66% DTP.*

To summarize, these results showed that our model was appropriate for the experimental values. Changes in composition of the fat mixtures affected the concentration of carotenes, which verifies our main hypothesis that fatty acid profile affects the diffusivity of carotenes in fat.

Diffusivity of carotenes increases with increased DTP concentrations. This effect was more pronounced with lycopene whose concentrations varied between 1.4 and 3.4 mg/ 100 mL when DTP increased from 2.22 to 6.66%.

B-carotene diffusivity reaches a plateau at a concentration of 4.44 % DTP (corresponding to a concentration of 2.7 mg β-carotene /100 mL). This saturation was not observed for lycopene, which could be explained by a competition between the two molecules. Lycopene is apolar with a linear carbon chain which makes its diffusivity higher in such a matrix compared to β-carotene [22].

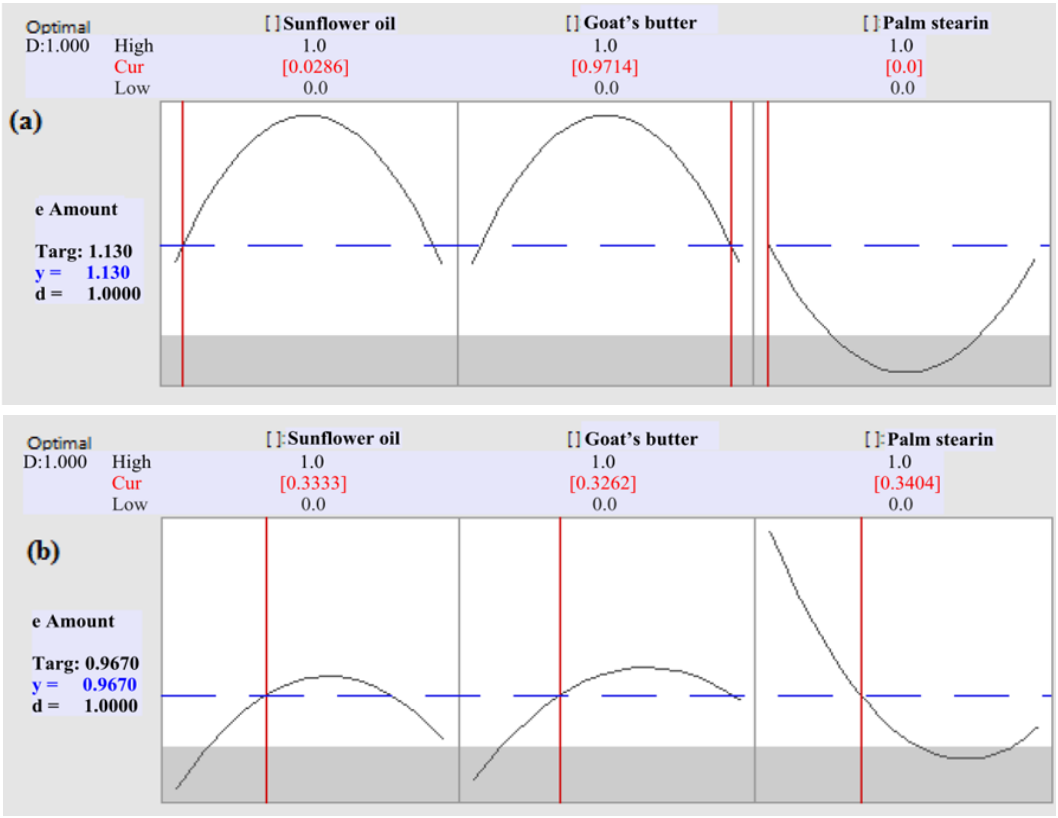

Figure 10. *Optimization diagram of diffusivity of lycopene (a) and β-carotene (b) in fat mixture with 2.22% DTP.*

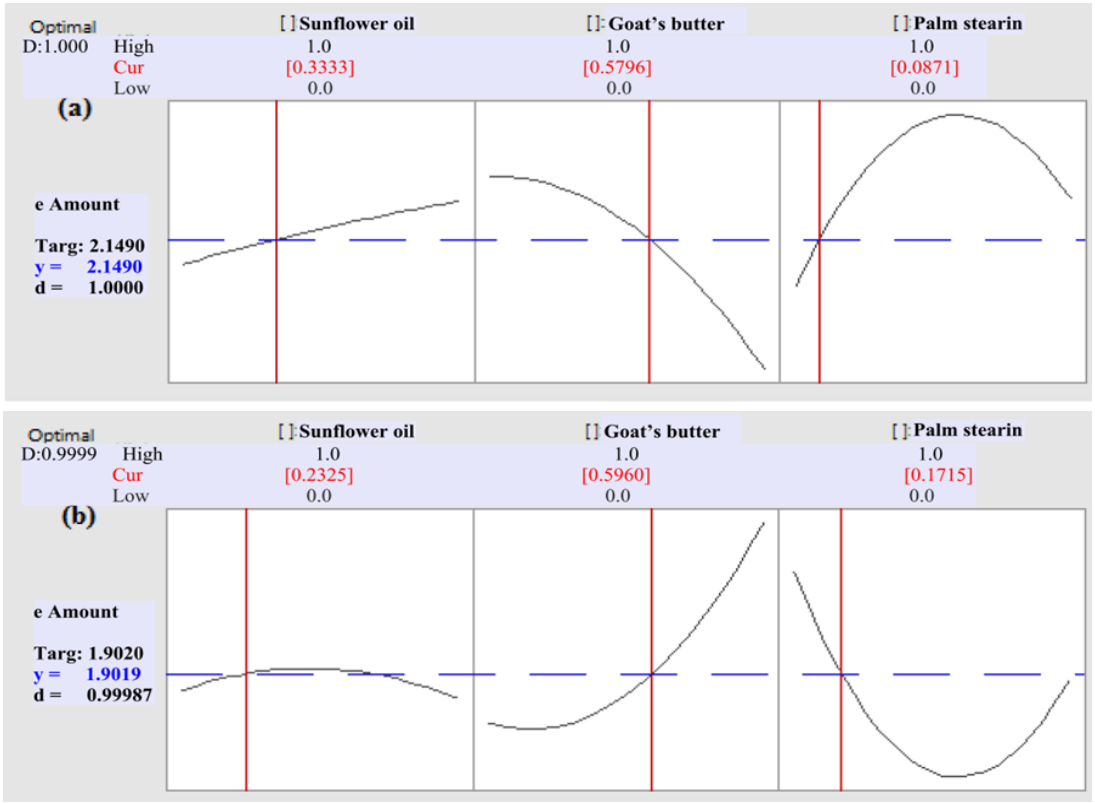

Figure 11. *Optimization diagram of diffusivity of lycopene (a) and β-carotene (b) in fat mixture with 4.44% DTP.*

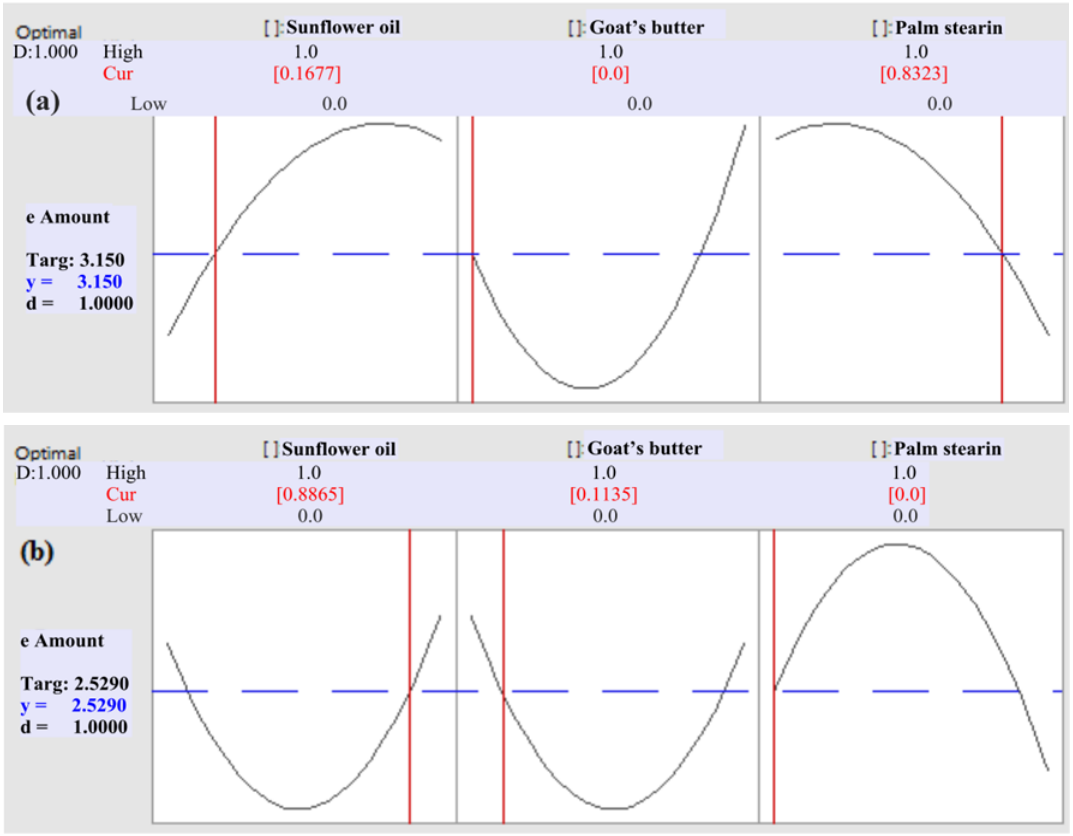

Figure 12 . *Optimization diagram of diffusivity of lycopene (a) and β-carotene (b) in fat mixture with 6.66% DTP.*

At 2.22% DTP, diffusivity of lycopene was optimal in equi-proportions of SO and GB. At higher concentration (4.44 % DTP), lycopene diffusivity is optimal in PS, and at 6.66% DTP, it is best in pure SO. The length of the fatty acid chains in SO and its low viscosity might facilitate lycopene diffusivity.

Diffusivity of β-carotene in GB at 4.44% DTP might be explained by its structure that contains two hexacycles, one in each extremity of the chain. This could reduce its apolarity and makes its diffusivity better in fat matrix with shorter fatty acids. With increased DTP proportion (6.66%) the effect of unsaturation could not be verified, which is in line with Borel's hypothesis [11].

With increasing DTP concentrations, lycopene diffusivity increases with fatty acid chain length, while the diffusivity of β-carotene is not associated directly with the fatty acid chain length. Its pattern might be due to other factors of competition and molecular structure.

IV. Conclusion

This study confirmed the importance of fatty acid profile in the absorption of carotenoids by affecting their diffusivity in the fat matrix. Diffusivity of lycopene was highest in fat matrix rich in long chain fatty acids and with the highest DTP incorporated (6.66%). At lower DTP concentration, lycopene diffusivity was higher with short chain fatty acid. The mechanism involved in the inversion in affinity of lycopene with increased DTP concentration is unclear. B-carotene diffusivity has a different pattern, it reaches a plateau at 4.44% DTP. Our study showed that unsaturation degree has no effect in the carotenes diffusivity in general.

For food technology applications, preparation including carotenes should take this model in consideration. For optimum diffusivity of lycopene a mixture should contain 82.23% PS and 16.77% SO while for a better diffusivity of β-carotene, a mixture of 88.65% SO and 11.35% GB is ideal. Further research is needed to elucidate the effect of polarity and viscosity of oils on carotenoids diffusivity; and patterns of competition between molecules and saturation of the fat matrix.

V. References

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