

Vegetable Substitutes Waxes

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Abstract. Natural vegetable waxes (esters of monocarboxylic fatty acid with primary monocarboxylic fatty alcohols, both saturated with linear hydrocarbonated chains) widespread in composition of horticultural products, with a major role in their physiology as a barrier to controlled transfer of water and other metabolites. Extruded products can be found on the foliar surface of fruits (pruine, suberine, cutine, etc.) where they exercise a protective function. The range constantly ascending to the large scale application as a film requires the diversification of the graded range by accessing the synthetic substitutes that requires toxicity demands and also, thermal or mass transfer performances. Polyoxyethylene chains with variable oligomerisation degree grafted with alcohols (phenols) with medium hydrocarbonate chain (C_8 , C_9) can be esterified with fatty acids (C_{16} , C_{18}) saturated and / or polyunsaturated obtained through the ecological integrated recovery of seed material from tomatoes processing [$C_{14}(0A)$ (6,34%); $C_{16}(0A)$ (31,6%); $C_{18}(0A)$ (21,15%); $C_{18}(1A)$ (40,81%)] grown in areas from west of Romania. Lipidic fraction was processed by solid/liquid repeated extraction with petroleum ether ($p.f.=40-60^{\circ}C$) (Soxhlet method) and classic analytical evaluated (refractive index, specific weight, iodine, saponification index) and gaschromatographic (column). "Homogeneous" polyoxyethylene chains with medium/large oligomerisation degree ($n=3,9,18$) processed by the Williamson method "step by step" from dyethyleneglycol, further grafted to 2-ethyl-hexyl alcohol respectively nonylphenol, were purified and characterized chemically and physical - chemical (colloidal).

Keywords: vegetable waxes, pruine, suberine, cutine, polyoxyethylene chain

INTRODUCTION

Preharvesting deprivations quantum of fresh fruits and vegetables is estimates at 25 – 80% depending of product and technological nature of operations. The number reflects the deficiency of professional competences regarding biological and environmental factors involved in degradations or the absence of adequate post harvesting technologies for maintaining fresh fruits and vegetables quality. Lately, it was given an *important attention to the study of superficial membrane potential of fruits and vegetables* involved in maintaining of fresh harvested product quality and to reduce the quantum of material packaging waste not biodegradable. Many fruits develop a superficial waxy film during ripening (apples, bananas, mango and grapes) with or without farinose aspect. The wax develops beginning with the second third of the development period of the fruit. The natural film is not able to offer enough protection against moisture loss at the high rate of fruit respiration. Because of this, prolonging "commercial life" of fruits and vegetables implies the restriction of respiration rate and the prevention of moisture loss for maintaining the vital nutritive elements in a quantity and quality closed to the fresh harvested products. The major importance and the continuous appreciation of fruits and vegetables in commerce headed to the development of

some techniques of waxing with or without fungicides, bactericides, growth regulators, showing a high potential of storage and transportation of these fresh products. Superficial films were intensely used for changing the internal atmospheric composition, drying delay, reducing the loss of moisture and a better commercial aspect.

Post harvesting loss can be reduced up to defined proportions, monitored through species selection, protective manipulation, cleaning and sorting, the control of maturation process, depravation, adequate packing with accessing the edible films, usage of ecological fungicides, ethylene absorbents, germination inhibitors, growth regulators, irradiation, storage operations, depositing, specific washing and waxing.

Fruits and vegetables waxing is recommended exclusively according active European recommendations and normatives. The classes of alimentary waxes are used to replace some natural products (waxes) removed during washing and cleaning operations, ameliorating the moisture loss during manipulation and commercialization. After waxing, the horticultural system is well dried before further manipulations.

Epicuticular wax fulfils minimum two major conditions: fruit surface hydrophobia and water permeability limitation, qualities that affects storage, distribution and retention of phytopharmaceutical products applied on fruits as solutions or emulsions. Permeability becomes a major problem when water soluble materials (calcium) must be introduced in fruits to produce imposed effects (preservation and firmness, texture). Although natural waxes are efficient in prevention of moisture loss, supplementary depositions of commercial waxes may ameliorate much more these losses contributing to storage prolong. While the fruit ripens, pelicular waxes grow uniform. After harvest, the quantity and the quality of natural waxes can change during storage time. Waxes biosynthesis is very influenced by environmental factors. Therefore is not surprising that during storage, the conditions can generate post harvesting changes of quantity and composition of protective waxy film. Very important is the fact that can be a large variety of differences in these changes. In some cases there are sensible changes in the quantity and composition of wax, while in other cases can be observed considerable modifications with notional deprivations of fruit aspect. The leaves generate abundantly wax, so that warming in a small quantity of water can rectify **5 – 10 g** waxy material from each leaf. Natural waxes used for fruits waxing are a complex of fatty materials and they contain some components that are found in apple wax too.

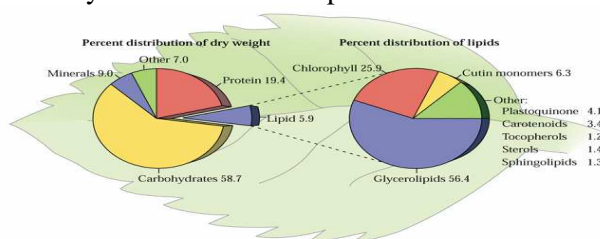


Fig. 1. Cuticular wax composition

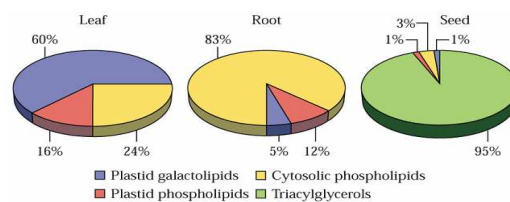


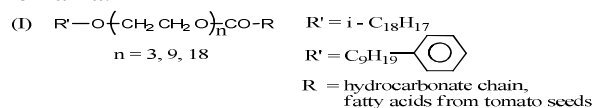
Fig 2. Lipids repartition on vegetable organs

A major difference between natural and synthesized wax is the **ursolic acid** (major component of apple wax) that is not present in carnauba or shellac wax. There are also other differences less important in chemical composition and some of them can be very relevant for obtaining the wanted aspect of the fruit. Wax quantity applied on fruits seems unimportant compared to natural existing wax. A careful study of the quantity of added wax in waxing processes confirmed that modification of layer thickness is not significant.

In 1994, FDA has regulated the use of waxes demanding that fruit processors and sellers to offer informations about the presence of waxes on fresh fruits and vegetables on the labels of individual products or on packages. The information should contain:

- covered with animal wax for freshness maintaining.
- covered with vegetable, oil, bee wax and/or based on shellac or resin wax for maintaining the freshness.

If one of these wax types is applied on fruits or vegetables, the label can notice this simply “*vegetable wax*”. *FDA* also allows the notifications “*not covered with wax or resins*” on fresh fruits and vegetables that do not contain such waxes. The constant growing interval regarding waxes applying as protective films on fruits and vegetables imposed in the presented study the diversification of wax types by accessing synthetic replacements that could correspond to toxicity exactingnesses and also the performances of thermal and mass transfer. The “*homogenous*” polyoxyethylene chains with variable oligomerization degree ($n = 3, 9, 18$) grafted on alcohols (phenols) with medium hydrocarbonated chains ($C_8; C_9$) can be esterified with saturated fatty acids ($C_{16}; C_{18}$) ($C_{16}; C_{18}$) and/or polyunsaturated acids obtained by integrated ecological refinement of seed material from tomatoes processing [$C_{14}(0A)$ (6,34%); $C_{16}(0A)$ (31,6%); $C_{18}(0A)$ (21,15%); $C_{18}(1A)$ (40,81%)] cultivated in areas from the west side of Romania.



The fatty fraction was processed by repeated solid/liquid extraction with petroleum ether ($p.f.=40-60^{\circ}C$) (Soxhlet method) and evaluated analytical classic (refraction index, specific weight, iodine index, saponification index) and gaschromatographic (column). The “*homogenous*” polyoxyethylene chains with medium/high oligomerization degrees ($n=3,9,18$) processed with *Williamson „step by step”* method from diethyleneglycol, grafted further on 2-ethyl-hexylic alcohol, respectively nonylphenol, were purified and chemically and physico - chemical characterized (colloidal).

MATERIALS AND METHODS

Materials

- tomato seeds (*Solanum lycopersicum*), byproduct from peocessing of horticultural materials, cultivated in Experimental Didactic Station of Banat’s University Of Agriculture Sciences and Veterinary Medicine Timișoara;
- nonylphenol (Sigma Aldrich) CAS 25154-52-3;
- 2 – ethyl hexyl alcohol (3 – octanol) (Sigma Aldrich) CAS 20296 – 29 – 1;
- triethylene glycol p. Tosylate (Sigma Aldrich) CAS 19249 – 09 – 7;
- triethylene glycol (Sigma Aldrich) CAS 112 – 27 – 6;

Installation :

- HPLC;
- Solid/liquid extraction plant;
- Esterification plant with standard appendix.

Methods (selective presentation)

Warm solid/liquid extraction of lipid fractions from tomato seeds

In a porcelain mortar are weighed tomatoes seeds (by difference), *with precision of 0.01 g, 10 – 15 g*, than are added *15 g of anhydrous Na_2SO_4 (Na_2CO_3)* and they are quantitatively transferred with a spatula in an *extraction cartridge (Soxhlet device)*. The mortar, the triturator and the spatula are cleaned with a cotton pad imbued with extraction solvent, also introducing further the cleaning cellulosic material in the cartouche. The extraction device is covered with clean cotton and it is fixed on the warming system. The

solvent is introduced (extraction benzene, oil ether with *p.f.*=30–60°C or n-hexan) up to complete siphon off and there are added more **50 mL** solvent. There are achieved **8 - 10 siphons/hour** in a period of **6 hours** at the reflux temperature, after which the cartouche is dropped off and the solvent is recovered. The clear, without impurities *miscela* is supplementary filtrated in a tarred balloon. **The purified fatty vegetable fraction** is exhaustive dehydrated **30 minutes** at **105°C in stove**, than is chilled in an exicator and weighted. If necessary, the drying and weighting operations are repeated up to constant weight. To facilitate the complete discharge of solvent remains, it is sparged a nitrogen flow (**8-10mL/min.**). The content of fatty vegetable fraction in the sample is evaluated with following ratio:

$$\text{Fatty vegetable fraction} = \frac{m_2 - m_1}{m_3} \cdot 100 (\%)$$

where: m_1 – empty balloon mass, (g);

m_2 – balloon mass with fatty vegetable fraction, (g);

m_3 – the mass of the evaluated sample, (g).

Cold solid–liquid and/or liquid/liquid extraction of lipid fractions from tomato seeds

Tomato seeds recuperated adequately are separated from impurities, are washed and carefully dehydrated in hot air flow (**25–40°C**), (during a variable period depending of the quantity), until the humidity goes below **1%**, the *marc* remains are separated, then the seeds are branched manually or automatically in a grinder, triturated with quartzous sand (SiO_2) until is obtained an “**homogeneous**” mass that is suspended by active agitation in a **Berzelius** glass of **500 mL** (or an adequate capacity to the mass of horticultural processed material) in **15–20 minutes**. After clarification the sand is repeatedly washed with water (integral separate recuperation of vegetal material and organic extracts). Aqueous reunited phases are introduced in separation cone with adequate capacity provided with a system of tight closing. It is added the adequate volume of oil ether (*p.f.*=30–60°C), extraction benzene (neofaline) (*p.f.*=40–60°C) or ethylic ether (1/1 ratio), it is active agitated by cone rotation at 180° and repeated depressurization of the system. The phases are separated and the operation is repeated **2–3** times with similar solvent volumes. **Organic reunited phases** are filtered over anhydrous Na_2CO_3 for complete discharge of water remains, then are distilled first at atmospherically pressure and further in advanced vacuum 10^2 – 10^3 mm col. Hg for complete discharge and recover of extraction solvent. Rough fatty fraction obtained is characterized qualitatively and quantitatively with chemical experimental techniques and specific instruments.

Total hydrolysis (HCl) of raw lipid fractions extracted from tomato seeds

In a processing glass pot, provided with mechanical agitator, ascendant refrigerant (reflux), thermostatic warming system, thermometer and weeping cone, is introduced **0.5 moles** saponificable fatty fraction and further under continuous agitation during **30 minutes** **0.6 moles HCl** concentrated (**35%**). After one hour of processing at **100–110°C**, the heterogeneous obtained mass is chilled at **40°C** and neutralized under active agitation with sodium hydroxide fine divided up to pink color of phenolphthalein (**it will be avoided excessive neutralization to prevent the saponification of superior acids**). Anorganic salts that result are filtrated in a **Büchner** cone, the eventually suspensions are discharged and the processing purified organic rough mass is extracted repeatedly in liquid–liquid system with anhydrous ethylic ether (**2–3 times**). **Organic reunited phases** are filtered over Na_2CO_3 are distilled first at atmospherically pressure and further in advanced vacuum (10^2 – 10^3 mm col.Hg). The isolated superior acids were chemical, physical–chemical and instrumental characterized with specific methods.

Preparation of “homogenous” hexaoxyethyleneglycol

In a processing pot provided with mechanical agitator, thermometer, weeping cone,

inert atmosphere are introduced **508 g (4 moles)** monosodium diethylenglicolate and then carefully **3,4–6g (2.2 moles)** 1,8–dichlorine–3,6–dioxo–octane, warmed at **90–95°C (around 72 hours)**, the processing mixture is neutralized with about **27mL** alcoholic solution of **NaOH 30%**. The processing mixture that contains “**homogenous**” hexaoxyethylenglicol is purified by repeated extractions in ethylic ether/water system. The main characteristics of **PEG-6** are reflected in the **table 1**. The efficiency reported to introduced diethylenglicol ranges between **50–60%**.

Tab. 1

Main characteristics of „homogeneous” polyethyleneglycols (n = 3,9,18)

No.	Symbol	Ethylene oxide contain ¹⁾ (%)		Hydroxyl values		Refractive index (n_D^{40})	
		experimental	calculated	experimental	calculated	experimental	calculated
1.	PEG-3	87.204	88.000	370.294	373.330	-	-
3.	PEG-9	94.772	95.650	130.024	135.265	1.4593	1.4591
4.	PEG-18	97.080	97.770	68.639	69.135	1.4626	1.4628

¹⁾ Determined by hydrogen iodide segregation

The preparation of “homogenous” nonaoxyethylenglicol

In a processing pot provided with mechanical agitator, thermometer, weeping cone, inert atmosphere refrigerant are introduced carefully at **110 - 130°C** during **2 hours 107.3 g (0.62 moles)** the monosodium salt of triethyleneglycol, **43.7 g (0.306 moles)** 1.8 – dichlorine - 3.6 – dioxo – octane and the mixture is warmed up for **5 hours at 150°C** until the negative reaction for phenolphthalein is present. The “**homogeneous**” nonaoxyethylenglycol is purified by repeated extractions in ethylic ether/water system. The main chemical, physical – chemical characteristics of **PEG-9** are presented in **Tab. 1**. The efficiency reported to introduced triethyleneglycol ranges between **50 – 55%**.

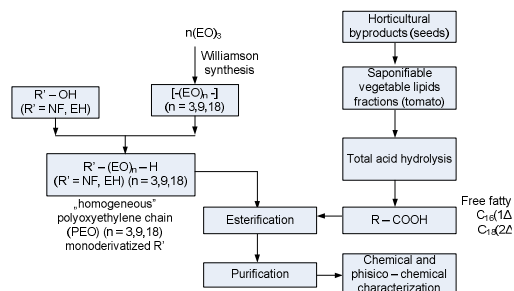


Fig. 3. Scheme for structuration of vegetable substitutes waxes

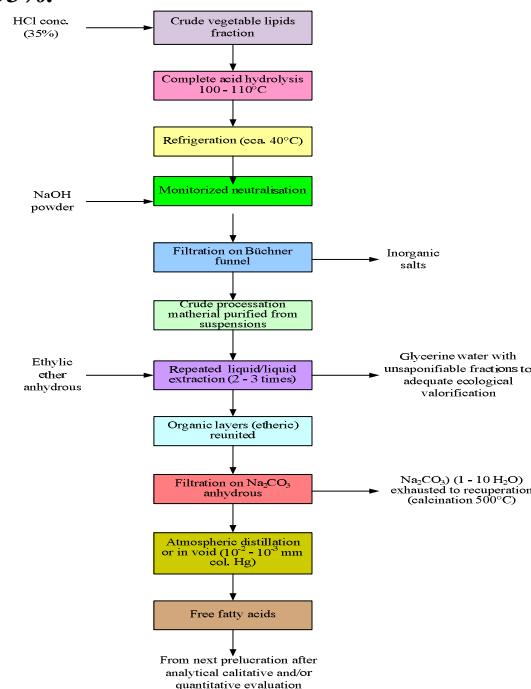


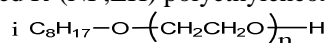
Fig. 4. Block scheme in the process of total acid hydrolysis with hydrochlorine acid of raw lipid fractions obtained by extraction from tomato seeds

The preparation of “homogenous” 2-ethyl-hexilic-polyethoxylated alcohol (n = 3)

In a processing pot provided with mechanical agitator, thermometer, inert atmosphere are dissolved **37.4 g (0.11 moles)** 2-ethyl-hexil tosylate, in **100 mL** anhydrous toluene, it is added carefully the solution of **17.4g (0.1 mols)** monosodium salt of homogenous triethyleneglycol in **50 mL** anhydrous toluene, the mixture is warmed up in reflux for **5–6 hours** (until the negative reaction for phenolphthalein is present) and the toluene is beared away on the bath (**18 - 25°C / 10 – 20 mm col. Hg**). The result is a consistent product, fluid, colored in pale yellow which is distilled (**10⁻²–10⁻³ mm col.Hg**) to obtain the homogenous polyethoxylated 2-ethyl-hexilic alcohol (**n=3**). The main chemical, physico–chemical characteristics are presented in **table 2**. The efficiency reported to alcohol ranges between **52–58%**. Similarly were prepared the alcohols 2-ethyl-hexilic “homogenous” polyethoxylated (**n=6.9.18**), characterized in **table 2**.

Tabl. 2

Main chemical and/or physic – chemical features of some „heterogeneous” (n = 3,9,18) monoderivatised R'(NF;EH) polyethyleneoxy (PEO) chains

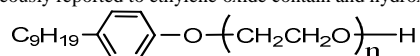


No.	Oligomerization degree (n)		Ethylene oxide contain ¹⁾ (%)		Hydroxyl values (mg KOH/g „homogeneous” polyoxyethylene (PEO) chain)		Purity ³⁾ (%)
	determined	calculated	determined ¹⁾	calculated	determined ²⁾	calculated	
1.	2.97	3	50.02	50.38	212.20	213.74	99.28
2.	8.95	9	74.73	75.29	105.66	106.46	99.25
3.	18.25	18	85.38	85.90	60.38	60.74	99.40

¹⁾ segregation with hydrogen iodide (HI) (d=1.8)

²⁾ acetylation with acetic anhydride in excess and acid – base titration of excess with alcoholic solution KOH 0.1n.

³⁾ weighted average value simultaneously reported to ethylene oxide contain and hydroxyl index.



No.	Oligomerization degree (n)		Molecular weight (M)	Ethylene oxide contain ¹⁾ (%)		Hydroxyl values (mg KOH/g „homogeneous” polyoxyethylene (PEO) chain)		Purity ³⁾ (%)
	determined	calculated		determined ¹⁾	calculated	determined ²⁾	calculated	
1.	2.99	3	352	37.41	37.50	158.69	159.99	99.75
2.	8.96	9	616	64.01	64.29	90.52	90.91	99.57
3.	17.95	18	1012	78.05	78.26	55.19	55.34	99.73

¹⁾ segregation with hydrogen iodide (HI) (d=1.8)

²⁾ acetylation with acetic anhydride in excess and acid – base titration of excess with alcoholic solution KOH 0.1n.

³⁾ weighted average value simultaneously reported to ethylene oxide contain and hydroxyl index.

RESULTS AND DISCUSSION

To achieve the proposed objectives the operating strategy was structured in three major phases (Fig. 2): the processing of homogenous polyoxyethylenic chains (**n=3.9.18**) monodrified **R(NF;EH)** accessing adapted **Williamson** method; the processing (extraction) and characterization of superior acids from tomatoes seed material (*Solanum lycopersicum*) (Soxhlet method); the proper processing of vegetable waxes substitutes as esters of nonilphenol (NF)/polyethoxylated 2-ethyl-hexanol (EH) (**n=3,9,18**) with fatty acids from the extracted oil purified and physico–chemical and chemical characterized from the seeds of tomatoes (**table 3**). Tomatoes seeds (*Solanum lycopersicum*), a subproduct obtained from tomatoes processing after complete separation of other impurities are dried and prepared for solid/liquid extraction. Because they have a high content of unsaturated superior acids (around **80%**), tocopherols (**120–150 mg%**), tomatoes oil is a quality superior fatty fraction, with a

real dietetic and anticholesteroleminate potential. Processing refuses consisted in seeds and peels differentiated quantitatively and qualitatively depending on: genus, culture and processing technology (2–4% peels with seeds); mixture of seeds, peels and pulp (25–35% raw material); in tomatoes paste processing from peeled tomatoes were obtained 10–20% peels, partial with adherent pulp. Simultaneous at selection resulted, depending on raw material and the way of peeling between 10–40% inappropriate peeled tomatoes which, usually are transferred in paste processing; at filed tomatoes canes processing resulted as waste the superior part of peduncle and the core with seeds, transferred also in paste technology. Using the tomatoes seeds for industrially oil extraction are still limited because of the small quantities of seeds obtained in processing.

The average composition of studied tomatoes seeds was: **humidity 5.8–7.7%, proteins 28.4–31%, fats 36–37.9%, raw fiber 21–28.2%, ash 3.4–4.3% and total sugars 2.3–3.5%**, lyzine content 5–6.5 g/16 g N, respectively methyonine and cystine 2.8–5.3 g/16gN. The resulted oil consisted in the following acids: stearic, oleic, linoleic (the most) and linolenic. The characteristics of tomatoes seeds oil studied in this paper were similar to other comestible oils characteristics (Tab. 3). The oil extracted from tomatoes seeds contained: **52,4–55,5%** linoleic acid with a ratio of superior saturated:unsaturated acids of **3,7:4,1**.

Tab. 3

Physical – chemical values and fatty acids composition from tomato seed oil

No.	Characteristics	Bibliography					
		[6]	[2]	[3]	[4]	[5]	[1]
1.	Density (g/mL)	0.909	0.921	0.916	0.920	0.919	0.918
2.	Cinematic viscosity (25°C) (CST)	47.12	49.04	49.58	48.83	49.28	50.15
3.	Dynamic viscosity (25°C) (CP)	46.18	47.03	44.82	45.58	45.39	45.21
4.	Ester value (mg KOH/g oil)	187.02	189.58	183.25	190.38	191.33	183.57
5.	Refractive index	1,466-1,468	1,4780-1,4793	1.4740	1,4720	1,4715	1,4672
6.	Saponifiable index (mg KOH/g oil)	186-194	190-192	182.40	191,50	190.48	184.15
7.	Iodine value (g iode/100 g oil)	112-125	118.63	116.30	120,50	119.75	118.34
8.	Unsaponifiable matter (%)	1,54	1,77	0.94	1.15	1.23	1.42
9.	Myristic acid (%)	-	1,02	1.49	-	1.10	0.92
10.	Palmitic acid (%)	13,02	9,56	15.34	12,62	9.58	10.34
11.	Stearic acid (%)	6,15	5,11	3.23	3,65	4.96	4.82
12.	Oleic acid (%)	46,28	33,45	30.82	20,12	28.53	43.19
13.	Linoleic acid (%)	35,44	49.25	48.75	61,63	53.26	36.28
14.	Linolenic acid (%)	-	2,57	0,33	2,14	2,54	2.93
15.	Total 9 – 14	100.89	100.96	99.96	100.16	99.97	99.90

From tomato seeds crude oil by treatment with hydrochloric acid were then removed phospholipids, then stoichiometric neutralized and distilled in vacuum 10^{-3} – 10^{-5} mm col.Hg. Glycerides resulted were hydrolysed and the fatty acids obtained by liquid / liquid extraction with petrol (*p.f.*=40-80°C) or petroleum ether (*pf*=40-60°C) and characterized chemical and physico-chemical. In the paper we considered necessary structuring the "homogeneous" polyoxyethylene chains ($n=3,9,18$) to increase the product mix of synthetic wax by direct modification of the HLB balance [1,2]. Esterification was carried out and monitored in classical laboratory equipment where yield was assessed by the amount of water collected in the process. After purification vegetable substituted wax have characterized chemical and physico-chemical. Wax emulsions subsequently obtained for the superficial coating of fruit were between 15-40% (weight) wax, superficialactive compound (emulsifiers) (5-25%) (sodium laurylsulphate) the rest is water. Color aqueous emulsions are preferred in organic solvents to remove the risk of accidental residual contamination, changes in fragrance, taste

and thermal degradation potential of some nutritional principles of fruit (**Tab. 4**). Protective efficacy ("**barrier**") of substitute vegetable wax is directly dependent on the size of "**homogeneous**" **polyoxyethylene** ($n = 3,9,18$) **chains** (hydrophilic) by which the hydrogen bond can hold water in the fruit construction with consequence of superficial saturation and limitation of mass transfer in both directions.

Tab. 4

Time dependence of film lemons weight with 15% substituted with vegetable wax, at 25°C, 2% sodium laurylsulphate (E-487).

Lemon	Weight loss (%) after							
	0	3	4	7	15	20	30	60
Film	-	2.85	3.98	5.32	8.54	10.63	15.40	20.82
Non film	-	2.85	4.75	5.95	9.83	13.26	20.82	35.03

CONCLUSION

Experimental results presented confirm the idea of diversifying the range of substitutes vegetable waxes with structures having "**homogeneous**" **polyoxyethylene** ($n = 3,9,18$) **chains** diderivatised with hydrocarbonated chains C_8 (**2 - ethyl - hexil**) C_9 (**nonylphenol**) respectively with unsaturated fatty acids, extracted from tomato seeds is beneficial both for toxicity, mass transfer performance and hydrophilic / hydrophobic balance. It requires extending the studies to other synthetic and vegetables / fruits waxes.

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