Encapsulated Antimicrobial Powder from Olive Fruit, Pomegranate and Orange Pomace Natural Aqueous Mix Extract using Advanced Homogenization Technology followed by Freeze Drying at Industrial Scale

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ABSTRACT

Natural antimicrobials is a new trend in Food induφstry with many applications against various food- and cosmetic-borne pathogen and spoilage microorganisms both bacteria and yeast & mold. Big food producers wordwide have committed themselves to substitute, in the short term, the conventional chemical antimicrobials, for example sorbates, benzoates etc., with natural antimicrobials which are not harmful and rather beneficial for human health. The majority of this novel natural antimicrobials contains sensitive natural polyphenols, flavonoids as active compound against pathogens and spoilage microorganisms, and therefore they must be treated by using novel low temperature processes in order to preserve their bio-actives. The most appropriate drying technology in order to achieve this is lyophilization. Thus in the context of the present experimental work, the preliminary step of high intension ultrasonic homogenization as well as the encapsulation drying step by lyophilization of a mixture of natural extracts (consisted by olive fruit extract, pomegranate pomace extract and orange pomace extract, are optimized. This optimization was carried out at industrial scale with target to obtain high added value and quality eco green antimicrobial powders to be used commercially against food- and cosmetic-bornε pathogenic and spoilage microorganisms.

Keywords: optimization of industrial production, ultrasonic homogenization, freeze drying, mixed natural antimicrobial extracts, olive fruit, pomegranate & orange pomace

JEL Classifications: C61, C93, C93, L66, O31

1. INTRODUCTION

Modern developments in the production of bio-active formulations from plant bio-wastes,have highlighted innovative technologies, as forming elements of the production line of the final high added value natural bioactive products**(REN Qilong et al.,2013).** These innovative technologies have the following characteristics:

- Maintenance of the desired bioactive properties and protection against oxidation of the final product by using low temperature and vacuum.
- Protection of the final product through micro- or nano-encapsulation techniques in appropriate edible carriers.
- Gradual release of the active substances and better bio-absorption resulting in better efficiency due to their use at lower concentrations.
- Masking of any unpleasant taste and odor

In a series of literature references, advanced cryogenic freeze drying is proposed to be used as a final stage for the production of dried encapsulated finished products **(AgnieszkaCiurzyńska&Andrzej Lenart,2011; Paul de Vos et al, 2010; Zuidam N.J &Nedovic V., 2010; V.Nedovic et al, 2011).** In addition, the initial mixing is suggested to be performed by modern ultrasonic technology that ensures the required high shear homogenization which guarantees the successful encapsulation of the active plant materials of polyphenols in the edible polymer that constitutes the encapsulating agent **(Chemat, F. et al , 2010 ; Mason et al, 1996).**
In the context of the present work, the optimization of the production of the novel natural antimicrobial products originating from mixed bio-wastes from olive oil, pomegranate juice and orange juice industry, was carried out by adopting the above mentioned novel technologies. The experimental production of the above mentioned products was carried out in two respective forms a) in the form of free flow powder in order to be used as potential natural anti-microbial product in food and cosmetics as well as b) in liquid, non-encapsulated form, for applications in organic or conventional plant protection. The experimental work was carried out by POLYHEALTH S.A and involved direct industrial scale development, in order to have available results directly applicable to the industrial production of the novel products without any need for scale up.

In the present study, extracts from solid wastes from orange and pomegranate juice industry obtained by using aqueous green vacuum microwave extraction at optimized operating conditions, were used as raw material for the production of liquid mixtures. These optimized extracts were concentrated to the maximum allowed concentration using spiral wounded reverse osmosis membranes, for their bioactive fortification via higher concentration, furthermore to reduce the load of the subsequent lyophilisation.
According to the research program, the reverse osmosis concentrates were used, and 71 liquid mixtures were produced by mixing them with MEDOLIVA, the liquid olive extract produced by POLYHEALTH S.A.
The mixing was carried out in appropriate proportions according to the pre-determined experimental plan of the proposal, and in excess of this in order to achieve more reliable results. In particular, instead of preparing 48 powders, a total of 71 powders were prepared and 48 of them with the highest total polyphenols content were used to be assayed for antimicrobial action. The purpose of mixing the three respective natural extracts was to take advantage of a potential synergistic antimicrobial effect of their bioactive components, according to the principles of hurdle theory in antimicrobial action as this is presented in the literature **(Leistner,L., et.al.(1995); C. Nichas, G. et.al.(2020)**

The following parameters were used as basic optimization parameters:
- ✓ Homogenization time in the ultrasonic homogenizer

- ✓ Mass ratio of active material and encapsulation material (% load of active material in the carrier)
- ✓ Type of water-soluble and clean label encapsulating material
- ✓ Total solid content in the solution to be lyophilized.
- ✓ The initial freezing temperature.
- ✓ The lyophilization cycle (time profile of vacuum and temperature) for the production of high quality finished product in powder form at high productivity (the lowest total lyophilization time).

In addition, in the context of the present work, 71 different combinations of the three liquid extracts -olive polyphenol, solid wastes of pomegranate juice and solid wastes of orange juice- were prepared in liquid form in order to be used as potential plant protection agents.

2. MATERIALS AND METHODS.

2.1.Freeze dryers

Two out of four industrial-scale lyophilizers, installed in the factory of POLYHEALTH S.A. in the area of Yannouli Larissa, were used to carry out the lyophilization experiments. These lyophilizers are constructed from stainless steel and were manufactured and supplied by the German company Zirbus technology GmbH with headquarters at HilfeGottes 1, 37539 Bad Grund (Harz), Germany.
The type of each lyophilizer used is EKS 100-10, with loading capacity of 100 KG of liquid material, divided in 44 loading trays and total square surface 10 m^2. The two lyophilizers are fully automated and operate using silicone heating/cooling liquid oil. The morphology of the above mentioned industrial scale lyophilizer is shown in Figure 1. which follows:

Figure 1. The morphology of the industrial type lyophiliator of POLYHEALTH S.A. which was used in the experimental project.

2.2. Ultrasonic homogenizer of continuous operation.

In order to achieve successful operation of the lyophilization process, a modern, of latest type continuous ultrasonic homogenizer was used. The principle of operation of this specific machine is to create high-intensity ultrasounds in a small aperture in the material circulation pipe.
The concentration of ultrasonic energy at this localized point results to high shear homogenization after re-circulation of the liquid for the appropriate time. The loading of the liquid is carried out continuously using a pump system. The ultrasonic machine also has a liquid cooling system and a recycling circuit. This advanced technology ultrasonic homogenizer, which is standard equipment of the natural antioxidant olive powder production line of POLYHEALTH S.A., has been supplied by the German company Hielscher Ultrasound Technology, Address: Oderstr. 53, 14513 Teltow, Germany phone: +49 3328 437-420 fax: +49 3328 437-444 email: info@hielscher.com and the type of the device is UIP1000hdT (1000W, 20kHz).
The morphology of the homogenization device is presented below in Figure 2:

Figure 2. The continuous ultrasonic homogenization device (type UIP1000hdT (1000W, 20kHz) supplied by German company Hielscher Ultrasound Technology.

2.3. The grinding mill for the Mixed Encapsulated powder of natural olive-rose-orange extracts.

In the context of the present research project, a state-of-the-art grinding mill was purchased by using funds from the research program. This state-of-the-art mill was used in order to grind the freeze dried solid material produced by the two lyophilizers, achieving satisfactory particle size reduction of the natural antimicrobial powder. The new grinding mill was purchased by the company Urschel Laboratories, Inc. 1200 Cutting Edge Drive, Chesterton, IN 46304 USA Telephone: + 219.464.4811. The type of the machine was: Comitrol® Processor Model 2100 and its morphology is shown in Figure 3 below

Figure 3. High-tech mill of Urschel Laboratories, Inc. for milling and powdering of lyophilized material.

2.4. UV-Vis photometer (UV-Vis)

A visible-ultraviolet (UV-Vis) photometer was used to determine absorption values during the experiments in order to optimize homogenization times of both liquid solutions and liquid natural extracts-maltodextrine solutions.The type of instrument used is Evolution 201 of Thermo Scientific and its morphology is given in Figure 4 below:

Figure 4. Spectrophotometer model Evolution 201 Thermo scientific.

2.5. Production Method of natural antimicrobials by mixing liquid solution of olive polyphenols and liquid extracts of industrial solid wastes of pomegranate and orange

Initially on the basis of the research program it was proposed to create 48 recipes with various proportions of liquid olive, pomegranate and orange extracts. Later in order to scan more effectively the best mixing ratios and obtain efficient liquid antimicrobials, it was decided to expand the number of mixtures to a total of 71 and to test

all of them for potential use in plant protection applications. Similarly, for the purpose of producing encapsulated powders from the mixed extracts for use against pathogenic and spoilage food-borne and cosmetic-borne microorganisms, 48 powders were selected out of the originally prepared 71 powders. The criterion which was used for the above selection was the highest concentration of total polyphenols in order to find among them the best acting powders against pathogenic and spoilage food- and cosmetic-borne fungi and bacteria. The prepared compositions of 68 liquid mixtures + 3 samples without mixing (olive, pomegranate, orange respectively), 71 in total), are summarized in Table 1 below:

TABLE 1.Composition of liquid mixtures of olive/pomegranate/orange extracts (% solids from each extract separately).

A/A	% LIQUID OLIVE FRUIT EXTRACT	% LIQUID POMEGRANATE POMACE EXTRACT	% LIQUID ORANGE POMACE EXTRACT	Total polyphenols in mg GAE/g powder
1	90	10	0	45,77 +
2	80	20	0	41,44 +
3	70	30	0	40 +
4	60	40	0	35,05 +
5	50	50	0	37,32 +
6	40	60	0	29,69 +
7	30	70	0	32,79 +
8	20	80	0	26,81 +
9	10	90	0	30,93 +
10	90	0	10	40,21 +
11	80	0	20	33,20 +
12	70	0	30	36,29 +
13	60	0	40	30,31 +
14	50	0	50	27,84 +
15	40	0	60	27,84 +
16	30	0	70	17,74
17	20	0	80	16,50
18	10	0	90	8,47
19	0	70	30	21,24
20	10	63	27	19,39
21	20	56	24	20,42
22	30	49	21	29,08 +
23	40	42	18	27,84 +
24	50	35	15	32,99+
25	60	28	12	37,11 +
26	70	21	9	39,17 +
27	80	14	6	41,03 +
28	90	7	3	48,66 +
29	0	50	50	16,50
30	10	45	45	13,21
31	20	40	40	20,21
32	30	35	35	16,50
33	40	30	30	27,43+
34	50	25	25	26,81 +
35	60	20	20	32,17 +
36	70	15	15	34,64 +
37	80	10	10	31,96 +
38	90	5	5	36,70 +
39	0	30	70	7,44
40	10	27	63	11,35

		TABLE 1. Continued		
41	**20**	**24**	**56**	14,65
42	**30**	**21**	**49**	**42,06 +**
43	**40**	**18**	**42**	**25,16 +**
44	**50**	**15**	**35**	**26,60 +**
45	**60**	**12**	**28**	**29,90 +**
46	**70**	**9**	**21**	**29,28 +**
47	**80**	**6**	**14**	**43,30 +**
48	**90**	**3**	**7**	**38,76 +**
49	**0**	**60**	**40**	11,56
50	**10**	**54**	**36**	15,89
51	**20**	**48**	**32**	17,33
52	**30**	**42**	**28**	**25,16 +**
53	**40**	**36**	**24**	**25,98 +**
54	**50**	**30**	**20**	**30,31 +**
55	**60**	**24**	**16**	**34,02 +**
56	**70**	**18**	**12**	**34,23 +**
57	**80**	**12**	**8**	**31,76 +**
58	**90**	**6**	**4**	**35,67 +**
59	**0**	**40**	**60**	10,94
60	**10**	**36**	**54**	14,65
61	**20**	**32**	**48**	18,98
62	**30**	**28**	**42**	21,04
63	**40**	**24**	**36**	**26,40 +**
64	**50**	**20**	**30**	**25,78 +**
65	**60**	**16**	**24**	**38,14+**
66	**70**	**12**	**18**	**38,56 +**
67	**80**	**8**	**12**	**37,53 +**
68	**90**	**4**	**6**	**33,61 +**
69	**100**	**0**	**0**	
70	**0**	**0**	**100**	
71	**0**	**100**	**0**	

+ Stands for the selected compositions of higher polyphenol values.

2.6.Total polyphenols determination method

For the determination of the total polyphenols as GAE (gallic acid equivalents) of the obtained mixed natural extracts, a slightly modified version of the method of **Singleton et al.(1999)and Waterhouse(2001)** was used. According to this method, initially a gallic acid solution was prepared by dissolving 0.5 g gallic acid in 10 mL pure ethanol, and the solution was then transferred in a 100 mL volumetric flask. The rest of the volume was filled by distilled water (preparation of a gallic acid stock solution of 5000 ppm). In addition, in a 1 L glass beaker, 200 g of anhydrous sodium carbonate were dissolved in 800 ml distilled water and the solution was boiled until the salt was fully dissolved. The solution was then cooled and kept for 24 h in dark for the formation of crystals of anhydrous sodium carbonate, which were removed by filtration the next day. The clear filtrate was finally dissolved in a total volume of 1 L by adding the remaining distilled water in a 1L volumetric flask. Consequently, a set of standards of gallic acid was prepared by diluting 0 mL, 1mL, 2mL, 3mL, 5mL, 10mL, and 20 mL of the gallic acid stock solution in six volumetric flasks of 100 mL each and filled with distilled water up to 100ml volume in order to prepare standard solutions of 0, 50, 100, 150, 250, 500 and 1000 ppm gallic acid. From each standard solution a quantity of 20 μL was mixed with 1,58 μL distilled water and 100 μL Folin Ciocalteu reagent in a glass tube and within 8 min a quantity of

100 μL sodium carbonate solution was added. After incubation for 2h at 20 ^{o}C, their absorbance was measured by a UV-Vis photometer (model EVOLUTION TM 201 supplied by Thermo-Scientific Co, Shanghai, China) against the blind solution (0 ppm gallic acid concentration). The standard curve depicting gallic acid concentration vs absorbance was constructed using the Microsoft Excel software and its R2 value was 0.9982. Calculation of the total polyphenols of mixed natural extracts was carried out following the same procedure and using the following equation of the standard curve:

Total polyphenol concentration of extract in ppm of GAE = Absorbance of sample at 765 nm / 0.001

(Equation 1)

Each measurement concerning total polyphenols was carried out in triplicate and the result was the average of the three obtained values.

3. RESULTS & DISCUSSION

3.1. Optimization of the homogenization process of the mixture of maltodexrin and mixed olive-pomegranate-orange extracts for the production of natural antimicrobial powders by lyophilization

In order to optimize the homogenization process of the mixtures of maltodextrin and mixed liquid natural extracts (olive-pomegranate and orange), a technique was used employing a continuous flow ultrasonic homogenizer. This homogenizer has been used for long time by POLYHEALTH S.A. to serve the production of its commercial product MEDOLIVA POWDER which consists solely of olive extract and maltodextrin DE 18. Furthermore, POLYHEALTH S.A. has developed a customized analytical technique, based on UV-Vis measurements, by which has already determined the optimum homogenization time

This technique is based on a measurement using the UV-Vis absorption of the liquid mixture of maltodextrin and mixed extracts at various times from the beginning of homogenization in order to determine the point at which the initially high absorption value, due to the presence of larger particles in the liquid, stabilizes and no longer changes significantly over time. Using this technique, a total of 51 homogenization experiments were carried out. During each one of them samples were taken every 5 minutes in a course of one hour, (i.e. a total of 12 samples for each type of mixture), and the corresponding minimum times required for absorption stabilization were determined and were recorded in Table 2 below. Furthermore, by observing the homogenization times in Table2., it is easily concluded that there is a short time range 35-40 min for the optimum homogenization times.

The above tests were carried out at maximum ultrasonic power and the cooling mechanism of the ultrasonic device was used to avoid potential degradation of the bioactive polyphenols. The solutions also contained the maximum percentage of maltodexrin i.e. 20 % (w/v) so that the homogenization times to be determined at this concentration would also be sufficient for effective homogenization at lower concentrations in the case that lyophilisation would give stable powders at lower proportion of maltodextin addition.

TABLE 2.Effective homogenization times of the various lyophilization recipies determined by using UV-Vis spectroscopy.

A/A	OLIVE	POMEGRANATE	ORANGE	TIME (min)	A/A	OLIVE	POMEGRANATE	ORANGE	TIME (min)
1	270	60	0	35	45	180	72	168	35
2	240	120	0	35	46	210	54	126	35
3	210	180	0	40	47	240	36	84	35
4	180	240	0	40	48	270	18	42	35
5	150	300	0	40	49	0	360	240	
6	120	360	0	40	50	30	324	216	
7	90	420	0	40	51	60	288	192	
8	60	480	0	40	52	90	252	168	35
9	30	540	0	40	53	120	216	144	40
10	270	0	60	35	54	150	180	120	40
11	240	0	120	35	55	180	144	96	40
12	210	0	180	35	56	210	108	72	40
13	180	0	240	35	57	240	72	48	40
14	150	0	300	35	58	270	36	24	40
15	120	0	360	35	59	0	240	360	
16	90	0	420		60	30	216	324	
17	60	0	480		61	60	192	288	
18	30	0	540		62	90	168	252	
19	0	420	180		63	120	144	216	40
20	30	378	162		64	150	120	180	40
21	60	336	144		65	180	96	144	35
22	90	294	126	40	66	210	72	108	35
23	120	252	108	40	67	240	48	72	35
24	150	210	90	40	68	270	24	36	35
25	180	168	72	40					
26	210	126	54	40	69	CONTROL1/OLIVE 100%			35
27	240	84	36	35	70	CONTROL2/ORANGE 100%			35
28	270	42	18	35	71	CONTROL3/POMEGRANATE 100 %			40
29	0	300	300						
30	30	270	270						
31	60	240	240						
32	90	210	210						
33	120	180	180	40					
34	150	150	150	40					
35	180	120	120	40					
36	210	90	90	35					
37	240	60	60	35					
38	270	30	30	35					
39	0	180	420						
40	30	162	378						
41	60	144	336						
42	90	126	294	35					
43	120	108	252	35					
44	150	90	210	35					

The results of Table 2 show that a homogenization time of 35-40 min is required for effective homogenization for all mixture compositions tested by lyophilization for the production of mixed powders for use as natural antimicrobials in food and cosmetics. The times given in Table 2 for each mixture separately were used for the homogenization of the individual liquid mixtures prior to their test as potential natural phyto-protectants.
More generally we could also say that homogenization of a total duration of 40 min at the maximum power of the ultrasonic homogenizer and with cooling operation activated to avoid potential degradation of the bioactive polyphenols of the mixture, is more than sufficient for all mixtures and is short enough, which means that it does not cause problems of delays in daily production.

3.2. Optimization of the mass ratio of active material to encapsulation material (maximization of the loading of the active material).

The mass ratio of encapsulant to bioactive materials is a significant parameter. By reducing this ratio the bioactivity of the produced lyophilized powder of the three natural extracts is increased. However, despite the fact this is something desired, certain limitations are arising when this is attempted, which are induced due to the reasons listed here below:

- The thermodynamic stability of the produced powder, or in other words the maintenance of its free flowing characteristics for the entire self-life and the avoidance of its transition to paste or even worse in hard compact form, is compromised at low encapsulant to bioactives mass ratio.
- The effective masking of the intense color and odor in the finished powder product, so that it can be used in food and cosmetics without acceptability problems, is also getting reduced at low encapsulant to bioactives mass ratio.

In order to determine the minimum addition of maltodextin to liquid mixed extracts, which can give stable and free flowing powders, experiments were carried out by testing maltodextrin additions of 5%, 10%, 15%, 20% w/v in the liquid mixture of the extracts. Consequently, the stability of 284 powders produced in this way by lyophilisation (4 × 71 samples=284 experiments) was examined twice on the day of their production and one month after been packed in a sealed plastic container. The results are presented in Table 3.

TABLE 3. Efficiency of production of stabilized free flowing lyophilized powders after addition of maltodexrin to the liquid mixtures of the natural extracts at various rates.

	COMPOSITION OF EXTRACT			ΠΡΟΣΘΗΚΗ ΜΑΛΤΟΔΕΞΤΡΙΝΗΣ			
A/A	OLIVE	POMEGRANATE	ORANGE	5%	10%	15%	20%
1	270	60	0	*	*	*	OK
2	240	120	0	*	*	*	OK
3	210	180	0	*	*	*	OK
4	180	240	0	*	*	*	OK
5	150	300	0	*	*	*	OK

		TABLE 3.Continued					
6	120	360	0	*	*	*	OK
7	90	420	0	*	*	*	OK
8	60	480	0	*	*	*	OK
9	30	540	0	*	*	*	OK
10	270	0	60	*	*	*	OK
11	240	0	120	*	*	*	OK
12	210	0	180	*	*	*	OK
13	180	0	240	*	*	*	OK
14	150	0	300	*	*	*	OK
15	120	0	360	*	*	*	OK
22	90	294	126	*	*	*	OK
23	120	252	108	*	*	*	OK
24	150	210	90	*	*	*	OK
25	180	168	72	*	*	*	OK
26	210	126	54	*	*	*	OK
27	240	84	36	*	*	*	OK
28	270	42	18	*	*	*	OK
33	120	180	180	*	*	*	OK
34	150	150	150	*	*	*	OK
35	180	120	120	*	*	*	OK
36	210	90	90	*	*	*	OK
37	240	60	60	*	*	*	OK
38	270	30	30	*	*	*	OK
42	90	126	294	*	*	*	OK
43	120	108	252	*	*	*	OK
44	150	90	210	*	*	*	OK
45	180	72	168	*	*	*	OK
46	210	54	126	*	*	*	OK
47	240	36	84	*	*	*	OK
48	270	18	42	*	*	*	OK
52	90	252	168	*	*	*	OK
53	120	216	144	*	*	*	OK
54	150	180	120	*	*	*	OK
55	180	144	96	*	*	*	OK
56	210	108	72	*	*	*	OK
57	240	72	48	*	*	*	OK
58	270	36	24	*	*	*	OK
63	120	144	216	*	*	*	OK
64	150	120	180	*	*	*	OK
65	180	96	144	*	*	*	OK
66	210	72	108	*	*	*	OK
67	240	48	72	*	*	*	OK
68	270	24	36	*	*	*	OK
69	CONTROL1 OLIVE EXTRACT 100 %			*	*	*	OK

	TABLE 3.Continued				
70	**CONTROL2 ORANGE POMACE 100 %**	*****	*****	*****	**OK**
71	**CONTROL3 POMEGRANATE POMACE 100 %**	*****	*****	*****	**OK**

(*)Stands for failure of the produced powder to stand free flowing
(OK)Stands for successful production of long term stable free flowing powder

We observed that with the addition of maltodextin in the extracts at:
- 5% w/v a sticky substance in all cases of extracts was produced instead of powder.
- 10% w/v the result was a slurry mass in all cases of extracts.
- 15% w/v the result was a powder which showed instability and for all extracts it was transformed after short time into a slurry and sticky mass that cannot be used for technological applications.
- 20 % w/v led to a successful production of stable, free flowing powder which, being packed in a properly sealed plastic container at ambient temperature, it was maintained for several months without any agglomeration or transformation into a sticky or glassy substance.

Therefore, we concluded that the optimal addition of maltodextrin DE18 to the liquid extract mixture, in order to obtain stable free flowing encapsulated powder from the natural extracts, is 20 % w/v.

3.3. Optimization of the total solid content in the solution to be lyophilized

The 20 % w/v solution of maltodexrin and mixed natural extracts has approximately 30% w/v total solid content. As showed above, if the percentage of maltodexrin is reduced, it is not possible to produce stable powder by lyophilization. Irrespectively the lyophilization conditions used, the composition of the created crystal does not provide a stable free flowing solid powder if the % of the maltodextrin added is reduced. However, since there is always the possibility of further dilution of the mixture with water to achieve a looser structure than that without adding water, four additional additions of water of 10, 20, 30, 50 % on the original solution were tested. At the end of lyophilization it was found that the produced powders had a loose structure and had no advantages over the powder produced without adding more water.
These tests were carried out on 10 random samples with compositions taken from Table 3
In conclusion, the optimal percentage of solids in the lyophilization solution is approximately 30% w/v resulting from the addition of 20% maltodextrin to the liquid mixture of extracts. This addition gives a finished lyophilized product with a soft structure and with higher productivity compared to the solutions which were further diluted with water. Further dilution with water does not give any advantage in texture and at the same time is connected with very low productivity and high energy consumption per kilogram of finished product. And this is due to the greater amount of water needed to be removed by lyophilization due to additional dilution.

3.4. Optimization of the initial freezing temperature and of the time profile of pressure and temperature during lyophilization.

The lyophilization cycle currently used by POLYHEALTH S.A. for the production of its commercial product, employs an initial freezing temperature of -35 °C. It was attempted to reduce it to -30 °C and even -20 °C with target to reduce the total time of lyophilization and

correspondingly the energy consumption per kilogram of finished product. A total of 15 representative tests were carried out with initial mixture compositions given in TABLE 4 below. By studying the results summarized in Table 4, it is concluded that the optimum initial freezing temperature is -35 °C and higher temperature is prohibited.

TABLE 4.Lyophilization tests with different initial freezing temperatures and their effect regarding the production of free flowing powders at the end of lyophilization.

	COMPOSITION OF EXTRACT					
A/A	OLIVE	POMEGRANATE	ORANGE	**-20 °C**	**-30 °C**	**-35 °C**
3	**210**	**180**	**0**	**NO**	**NO**	**SUCCESS**
4	**180**	**240**	**0**	**NO**	**NO**	**SUCCESS**
13	**180**	**0**	**240**	**NO**	**NO**	**SUCCESS**
15	**120**	**0**	**360**	**NO**	**NO**	**SUCCESS**
23	**120**	**252**	**108**	**NO**	**NO**	**SUCCESS**
27	**240**	**84**	**36**	**NO**	**NO**	**SUCCESS**
38	**270**	**30**	**30**	**NO**	**NO**	**SUCCESS**
45	**180**	**72**	**168**	**NO**	**NO**	**SUCCESS**
52	**90**	**252**	**168**	**NO**	**NO**	**SUCCESS**
54	**150**	**180**	**120**	**NO**	**NO**	**SUCCESS**
55	**180**	**144**	**96**	**NO**	**NO**	**SUCCESS**
68	**270**	**24**	**36**	**NO**	**NO**	**SUCCESS**
69	**CONTROL1 OLIVE EXTRACT 100 %**			**NO**	**NO**	**SUCCESS**
70	**CONTROL2 ORANGE POMACE 100 %**			**NO**	**NO**	**SUCCESS**
71	**CONTROL3 POMEGRANATE POMACE 100 %**			**NO**	**NO**	**SUCCESS**

3.5. Optimization of the lyophilization cycle

For the optimization of the lyophilization cycle, the cycle was broken into three stages:

FIRST STAGE: -35 °C to -10 °C and vacuum P= 0.15 mbar-0.5 mbar-1mbar
SECOND STAGE: -10°C to +5°C and vacuum P = 0.15 mbar-0.5 mbar-1 mbar
THIRD STAGE: +5°C to 43°C and vacuum P= 0.15 mbar-0.5 mbar-1mbar

Where the above temperatures correspond to the heating medium and the pressures reflect to three alternative vacuum values of the chamber (low-medium-high)

Regarding the product which undergoes lyophilisation, the overall target was to reach 43 °C at a maximum time of 2160 min (36 hours)

Initial TARGET -35 °C to 43 °C 2160 min (36 hours)

By rotation of the vacuum values of the three pre-defined stages of lyophilization,as shown in the Following Table 5, and by subsequent application of lyophilization at the determined conditions, the total lyophilization times were obtained and are listed in the last column of Table 5.

A study of the values,listed in Table 5, shows that the desired minimum total time for lyophilization is much shorter than 36 hours and equals to 21 hours, which results the optimal total time of lyophilization.

The material used for lyophilization was a mixture containing approximately equal amounts of the three extracts and 20 % w/v

maltodextin. The same result was then repeated and confirmed for all other compositions that were lyophilized to produce powders.

TABLE 5.Optimization of the total lyophilization time for the production of encapsulated powders of mixed olive-pomegranate-orange extracts with maltodextin DE18 carrier.

a/a	STAGE 1 vacuum in mbar	STAGE 2 vacuum in mbar	STAGE 3 vacuum in mbar	TOTAL LYOPHILIZATION TIME (in hrs)	
1	0.15	0.15	0.15	27	
2	0.15	0.15	0.5	26	
3	0.15	0.15	1	25	
4	0.15	0.5	0.15	27,5	
5	0.15	0.5	0.5	24,5	
6	0.15	0.5	1	24	
7	0.15	1	0.15	25,5	
8	0.15	1	0.5	23,5	
9	0.15	1	1	25	
10	0.5	0.15	0.15	25,5	
11	0.5	0.15	0.5	24,5	
12	0.5	0.15	1	24	
13	0.5	0.5	0.15	21 hrs OPTIMUM	
14	0.5	0.5	0.5	22,5	
15	0.5	0.5	1	23	
16	0.5	1	0.15	23,5	
17	0.5	1	0.5	26	
18	0.5	1	1	24,5	
19	1	0.15	0.15	23	
20	1	0.15	0.5	25	
21	1	0.15	1	24	
22	1	0.5	0.15	21.5	
23	1	0.5	0.5	23.5	
24	1	0.5	1	24	
25	1	1	0.15	23	
26	1	1	0.5	22.5	
27	1	1	1	26	

3.6. Optimization of the type of used water-soluble and clean label encapsulating material.

In addition to maltodextrin DE18, three more edible biopolymers were tested as encapsulating agents for the mixed solution of olive, pomegranate and orange extracts.
Specifically, b-cyclodextrin, guar gum, and WPC 80 cheese whey protein. The tests were carried out at small scale and it was found that there was a satisfactory production of free flow powders with all three of

these materials at 20% w/v, similar to that produced previously by using maltodextrin.
However, an attempt to reduce the addition of the above three carriers to 15%w/v, showed again that the produced product was in the form of a sticky mass and not in the desired form of free flowing powder.
In addition to the data shown in Figure 5. where typical wholesale prices of maltodextrin and alternative to maltodextrin encapsulants are presented, it is concluded that the price range between them is large and maltodextrin is the cheapest among them.

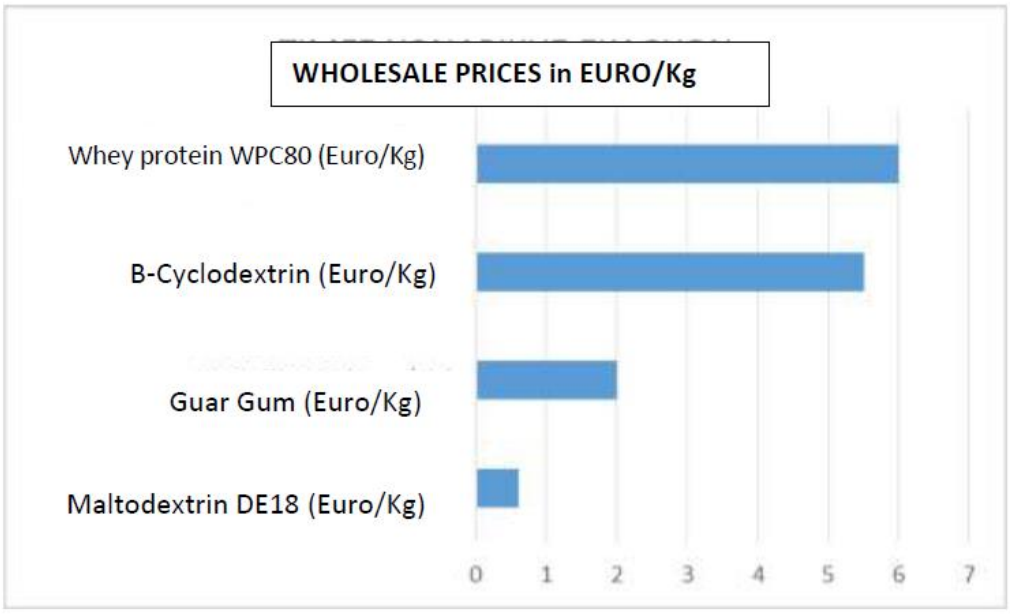

Figure 5. Wholesale prices of the various encapsulation carriers.

Based on the data presented in Figure 5., it is obvious that maltodexrin DE18 without falling short of advantages over other biopolymers is significantly cheaper and is, therefore, chosen as the most appropriate natural carrier for encapsulating the liquid mixture of the three mixed olive-pomegranate-orange natural extracts.

4. CONCLUSIONS

The results of the optimization procedures in the production of anti-microbial natural powders showed that:
a) The optimum homogenization time by ultrasounds is 40 min
b) The optimum addition of maltodextrin DE18 was found to be 20% w/v on the liquid mixture in order to create stable free flowing powders.
c) The best encapsulating agent, in operational and economic terms, was found to be maltodextrin DE18
d) The optimum content of total solids in the solution to be lyophilized was found to be 30% w/v
e) The optimum initial freezing temperature was found to be -35 °C. f) The optimum time profile of temperature and vacuum to achieve production of stable free flowing powders in the minimum time is: STAGE A: from -35 °C to -10 °C with vacuum of 0.5 mbar, STAGE B: from -10 °C to 5 °C with vacuum of 0.5 mbar , STAGE C: from 5 °C to 43 °C with vacuum of 0.15 mbar

g) The optimum total lyophilization time obtained by adopting the above mentioned lyophilization conditions was found to be 21 hours.

In addition, an important conclusion drawn from the measurement of the total polyphenols of 71 powders of mixed extracts produced, is that the relative ratios of the solids of the three extracts containing the maximum concentration of total polyphenols are: A. Olive solids: 90% Pomegranate solids: 7% Orange solids: 3% (sample No:28)
B.Olive solids: 90% Pomegranate solids: 10 %, Orange solids: 0 % samlpe No: 1)
C.Olive solids: 80% Pomegranate solids: 20% Orange solids: 0 % (sample No 2)
D.Olive solids: 80% Pomegranate solids: 14% Orange solids: 6 %.(sample No:27)
This means that the extracts which are most likely to act as natural anti-microbial powders are those that contain more olive polyphenols (80-90% of solids). Additionally, the relative composition of the solids of the pomegranate and orange extracts, despite their smaller proportion in the crystal, plays an important role in the final active concentration of total polyphenols which is determined by the Folin-Ciocalteu method. This observation is particularly important for the optimization, and justifies the multi-point optimization chosen in this paper which, in fact, could be applied more generally by POLYHEALTH S.A. to develop additional products in the future.

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