Development of High Added Value Encapsulated Antimicrobial Powders from Mixed Natural Aqueous Extract from Olive Fruit and Pomegranate & Orange Pomace by Advanced Homogenization Technology and Freeze Drying at Industrial Scale

Stavroula Lada*, Fani Karkanta and Dimitrios Charisis

University of Thessaly, Greece

*Corresponding Author

Abstract

Natural antimicrobials is a new trend in food and cosmetic industry with many applications against various food- and cosmetic-borne pathogens and spoilage microorganisms (bacteria as well as yeast & molds.) Big food and cosmetic producers worldwide 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 and flavonoids as active compounds 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, a system with a preliminary step of high intension ultrasonic homogenization followed by lyophilization is optimized in order to encapsulate the bio-actives of a mixture of three natural extracts (olive fruit-pomegranate pomace and orange pomace). 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 in food and cosmetic applications.

Keywords: optimization of industrial production, freeze drying , antimicrobial extracts, olive fruit extract, pomegranate & orange pomace.

1. Introduction

Modern developments in the production of bio-active formulations from plant bio-wastes,have highlighted innovative technologies, as forming steps of the production line of the finished high added value natural bioactive products **(REN Qilong *et al.*, 2013).**
On the other hand, 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 powders **Agnieszka Ciurzyńska &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 of the liquid products is suggested to be performed by modern ultrasonic technology that ensures the required high shear homogenization. This guarantees the successful encapsulation of the active plant materials of polyphenols and flavonoids in the edible polymer that constitutes the encapsulating agent **(Chemat *et al* , 2010 ; Mason *et al*, 1996).**
In the context of the present work, the optimization of the production of the novel natural antimicrobials produced by mixing olive fruit extract with pomegranate and orange extracts with encapsulant was carried out by employing the above mentioned advanced technologies. The experimental production of the products was in the form of free flowing encapsulated powder to be used as potential natural anti-microbial products in food and cosmetics. The experimental work was carried out by POLYHEALTH S.A and involved direct industrial scale development. This was done in order to have available results directly applicable for 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 extracts were initially concentrated to the maximum allowed concentration using spiral wounded reverse osmosis membranes, for their bioactive fortification at higher concentration, with purpose to reduce the load of the subsequent lyophilization. Finally, according to the research program, the reverse osmosis concentrates were used, and 68 liquid mixtures were produced by mixing them with MEDOLIVA, a commercial liquid olive fruit extract currently produced by POLYHEALTH S.A.

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,1995).**

Thus the main target of the present work is to optimize at industrial scale the following process parameters:

- ✓ The homogenization time in the ultrasonic homogenizer
- ✓ The level of addition of encapsulation material (% w/v addition of encapsulant in the liquid mixture of the three natural extracts)
- ✓ The type of water-soluble and edible encapsulating material
- ✓ 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 (prediction of the lowest total lyophilization time).

2. Materials And Methods.

2.1.Freeze dryers

Two out of four EKS 100-10 industrial-scale lyophilizers of 100 kg capacity, installed in the factory of POLYHEALTH S.A. in Giannouli Larisa, 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.

Figure 1. *The morphology of the industrial type lyophilizer*

Source: POLYHEALTH S.A.

2.2. Continuous Ultrasonic homogenizer.

In order to achieve successful operation of the lyophilization process, a modern, of latest type continuous ultrasonic homogenizer was used to homogenize the extracts with the encapsulant before freeze drying. The principle of operation of this specific machine is to create high-intensity ultrasounds in a small aperture in the material circulation pipe. The exact type of the used machine was UIP1000hdT (1000W, 20kHz) commercialized by Hielscher Ultrasound Technology, Address: Oderstr. 53, 14513 Teltow, Germany

2.3. The grinding mill for the Encapsulated powder of the mixed natural extracts.

In the context of the present research project, a state-of-the-art grinding 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

2.4. UV-Vis photometer (UV-Vis)

A UV-Vis photometer was used to determine absorption values during the experiments in order to optimize homogenization times of liquid natural extracts-maltodextrin solutions.The type of instrument used was Evolution 201 of Thermo Scientific Co, This photometer was also used for the determination of Total Polyphenols of the produced powders.

2.5. Production Method of natural antimicrobials powders.

Initially on the basis of the research program it was decided to create 48 recipes with various proportions of liquid olive, pomegranate and orange extracts. Later in order to determine more effectively the best mixing ratios, it was decided to expand the number of mixtures to a total of 68. Furthermore, for the purpose of producing encapsulated powders from the mixed extracts for use against pathogenic and spoilage food-borne and cosmetic-borne microorganisms, 48 compositions (figures marked with cross in Table 1) were selected out of the originally prepared 68 powders. The criterion which was used for the selection was the higher concentrations of total polyphenols in order to trace among them the best performing powders against pathogenic and spoilage food- and cosmetic-borne fungi and bacteria. The originally prepared compositions, 68 in total, are summarized in Table 1 below:

TABLE 1. Composition of liquid mixtures used to prepare antimicrobial powders and total polyphenols content of the powders.

A/A	% LIQUID OLIVE FRUIT EXTRACT	% LIQUID POMEGRANATE POMACE EXTRACT	% LIQUID ORANGE POMACE EXTRACT	Total polyphenols in mg GAE/g of powder
1	90	10	0	45.77 +
2	80	20	0	41.44 +
3	70	30	0	40.00 +
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
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	8.56
67	80	8	12	37.53 +
68	90	4	6	33.61 +

+ Stands for the selected compositions of higher polyphenol values. 48 in total

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 methods of **Singleton et al.(1999)** and **Waterhouse(2001)** was used. Each measurement concerning total polyphenols was carried out in triplicate and the result was expressed as the average of the obtained values.

3. Results & Discussion

3.1. Optimization of the homogenization process of the mixture of maltodextrin DE18 and mixed olive-pomegranate-orange extracts.

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. In particular, POLYHEALTH S.A. has developed a customized analytical technique, based on UV-Vis measurements, by which has already determined the optimum homogenization time for its commercial product

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 68 homogenization experiments were carried out. During each one of them, samples were taken every 1 minutes in a course of 10 min, and the corresponding minimum times required for absorption stabilization were determined. Furthermore, by observing the homogenization times , it was easily concluded that there was a short time range 3-4 min for the optimum homogenization times.

The above tests were carried out at maximum ultrasonic power (4 kW) and the cooling mechanism of the ultrasonic device was used to avoid potential thermal degradation of the bioactive polyphenols. The solutions also contained the maximum percentage of maltodextrin encapsulant 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 maltodextrin addition.

Thus, the results indicated that a homogenization time of 3-4 min is required for effective homogenization for all compositions that tested. More generally we could also say that

homogenization of a total duration of 4 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 level of maltodextrin addition in the liquid extract mixtures.

The mass ratio of maltodextrin encapsulant to bioactive materials in the liquid is a significant parameter as by reducing this ratio the bioactivity of the produced lyophilized powder of the three natural extracts is increased. However, despite the fact this is desirable, certain limitations are arising when this is attempted, which are induced due to the reasons listed 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 taste 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.

Therefore, in order to determine the minimum allowed addition of maltodextrin to the liquid mixture of extracts, which can give stable and free flowing powders, experiments were carried out by testing maltodextrin additions of 5%, 10%, 15%, 20% w/v to the liquid mixture of the extracts. Consequently, the stability as well as the color and taste masking of 272 powders produced by lyophilisation (4 compositions × 68 samples=272 experiments) was examined at two times: on the day of their production and second a month after been packed in a properly sealed plastic containers.

Following this procedure it was observed that with the addition of maltodextrin in the extracts at level:

- 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 into a slurry and sticky mass after short time that could not be used for technological applications.
- 20 % w/v led to successful production of stable, free flowing powder which, being packed in a properly sealed plastic container at ambient temperature, was kept for several months without any agglomeration or transformation into a sticky or glassy substance. In addition the powders produced with 20% addition of maltodextrin had similar color and taste like the commercial MEDOLIVA powder currently produced by POLYHEALTH S.A.

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

3.3. Optimization of the initial freezing temperature.

The lyophilization cycle currently used by POLYHEALTH S.A. for the production of its commercial product, employs an initial freezing temperature of -35 ^{o}C. It was attempted to reduce it to -30 ^{o}C and even -20 ^{o}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 2 below. By studying the results summarized in Table 2, it is concluded that the optimum initial freezing temperature is -35 ºC and higher temperature is prohibited.

TABLE 2. 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 ºC	-30 ºC	-35 ºC
3	70	30	0	NO	NO	SUCCESS
4	60	40	0	NO	NO	SUCCESS
13	60	0	40	NO	NO	SUCCESS
15	40	0	60	NO	NO	SUCCESS
23	20	56	24	NO	NO	SUCCESS
27	80	14	6	NO	NO	SUCCESS
38	90	5	5	NO	NO	SUCCESS
45	60	12	28	NO	NO	SUCCESS
52	30	42	28	NO	NO	SUCCESS
54	50	30	20	NO	NO	SUCCESS
55	60	24	16	NO	NO	SUCCESS
68	90	4	6	NO	NO	SUCCESS
69	CONTROL1 OLIVE EXTRACT 100 %			NO	NO	SUCCESS
70	CONTROL2 ORANGE POMACE 100 %			NO	NO	SUCCESS
71	CONTROL3 POMEGRANATE POMACE			NO	NO	SUCCESS

- SUCCESS stands for production of free flowing powder

-NO means production of melted or glass type material

3.4. Optimization of the lyophilization cycle

For the optimization of the lyophilization cycle, the cycle was broken into three stages:
FIRST STAGE: -35 ºC to -10 ºC and vacuum settings P= 0.15 mbar or 0.5 mbar or 1mbar
SECOND STAGE: -10ºC to +5ºC and vacuum settings P= 0.15 mbar or 0.5 mbar or 1mbar
THIRD STAGE: +5ºC to 43ºC and vacuum settings P= 0.15 mbar or 0.5 mbar or 1mbar
Regarding the product which undergoes lyophilization, the overall target was to reach 43 ºC at a maximum time of 2160 min (36 hours)
TARGET -35 ºC to +43 ºC in 2160 min (36 hours)
By rotation the vacuum values of the three pre-defined stages of lyophilization, as shown in Table 3, 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 3.

A study of the values listed in Table 3, shows that the obtained minimum total lyophilization time is much shorter than 36 hours and equal to 21 hours and it is obtained when the pressures of the three lyophilization steps are set at 0.5, 0.5 and 0.15 mbar respectively.
The material used for lyophilization was a mixture containing approximately equal amounts of the three extracts and 20 % w/v maltodextrin.

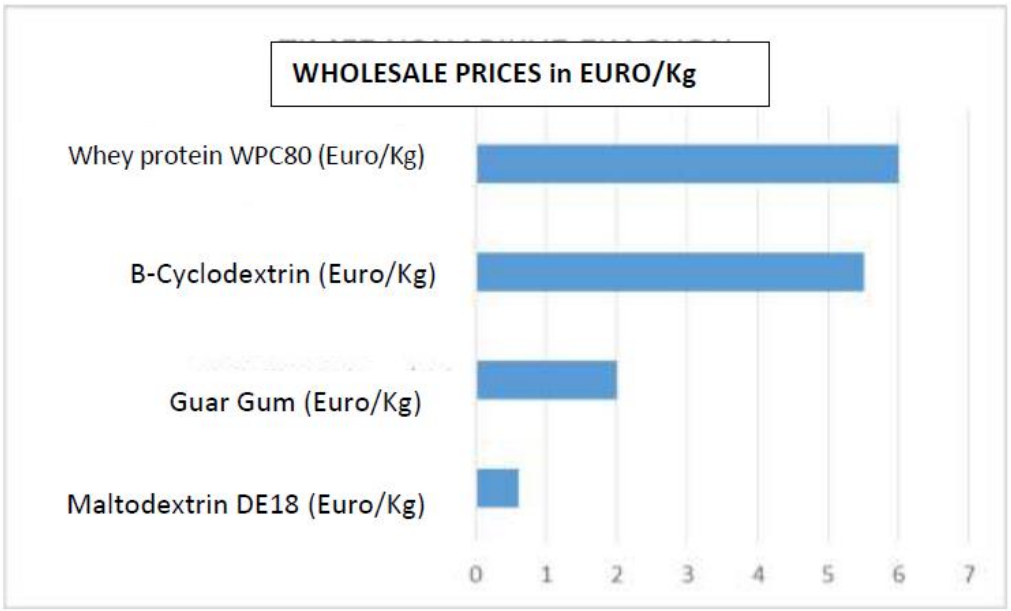
TABLE 3. Lyophilization times at various profiles of vacuum

a/a	STAGE 1 vacuum in mbar	STAGE 2 vacuum in mbar	STAGE 3 vacuum in mbar	TOTAL LYOPHILIZATION TIME (in hours)
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	21hours OPTIMUM VALUE
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

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

In addition to maltodextrin DE18, three 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 flowing powders with all three 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 finished 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 2. 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 2. Indicative average wholesale prices of the various encapsulation carriers.



Source :Market Research using internet

Based on the data presented in Figure 2., it is obvious that maltodextrin DE18 without falling short of advantages over other biopolymers is significantly cheaper and it is, therefore, chosen as the most appropriate natural carrier for encapsulating the liquid mixture of the three 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 4 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 minimum maltodextrin addition in the liquid natural extract mixture to obtain free flowing powders is 20% w/v ,Addition at lower levels lead to production of sticky material.
e) The optimum initial freezing temperature was found to be -35 ^{o}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 ^{o}C to -10 ^{o}C with vacuum of 0.5 mbar, STAGE B: from -10 ^{o}C to 5 ^{o}C with vacuum of 0.5 mbar , STAGE C: from 5 ^{o}C to 43 ^{o}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 68 powders of mixed extracts, 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 % (sample 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.

Acknowledgments: This research has been co- financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (Project code:T1EDK-03942).

References

REN Qilong, XING Huabin, BAO Zongbi, SU Baogen, YANG Qiwei, YANG Yiwen and ZHANG Zhiguo.(2013). "Recent Advances in Separation of Bioactive Natural Products," *Chinese Journal of Chemical Engineering*, vol.21(9), pp. 937—952. DOI: 10.1016/S1004-9541(13)60560-1

Ciurzyńska, A. and Lenart, A.(2011). "Freeze-drying today – applications and properties of freeze-dried food products".*Polish Journal of Food and Nutrition Sciences*, vol. 61(3), pp.165-171

DeVos, P., Faas, M.M., Spasojevic, M. and Sikkema, J. (2010). "Encapsulation for preservation of functionality and targeted delivery of bioactive food components," *International Dairy Journal*, Vol. 20(4), pp. 292-302.

Zuidam, N.J. and Nedovic, V. (2010). *Encapsulation Technologies for Active Food Ingredients and Food Processing*.Springer, New York.http://dx.doi.org/10.1007/978-1-4419-1008-0

Nedovic, V., Kalusevic,A., Manojlovic,V., Levic,S. and BrankoBugarski.(2011). "An overview of encapsulation technologies for food applications," *Procedia Food Science* Vol.1 (2011), pp.1806 – 1815 (presented at 11th International Congress on Engineering and Food (ICEF11)).doi:10.1016/j.profoo.2011.09.266

Chemat F, Zill-e-Huma, Khan M.K.(2011). "Applications of ultrasound in food technology: Processing, preservation and extraction. " *Ultrason Sonochem.Jul*; Vol.18(4), pp.813-35.doi:10.1016/j.ultsonch.2010.11.023.Epub 2010 Dec 16. PMID: 21216174.

Mason,T.J.,Paniwnyk,L. and Lorimer, J.P.(1996). "The uses of ultrasound in food technology," *UltrasonicsSonochemistry*, Vol.3 (1996), pp.253-260

Leistner, L. and Gorris, L.G.M. (1995). "Food Preservation by hurdle technology," *Trend in Food Science and Technology*., Vol.6, pp. 35-37.

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. (1999)."Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent." *Methods in Enzymology*. vol. 299, pp.152-178, https://doi.org/10.1016/S0076-6879(99)99017-1

Waterhouse, A.L.(2001). *Determination of Total Phenolics.* In: Wrolstad, R.E., Ed., Current Protocols in Food Analytical Chemistry. Ed. John Wiley & Sons, New York, I1.1.1-I1.1.8.