

# Production of Novel Bioactive Yogurt Enriched with Olive Fruit Polyphenols

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**Abstract**—In the course of the present work, plain (non-encapsulated) and microencapsulated polyphenols were produced using olive mill wastewater (OMW) as raw material, in order to be used for enrichment of yogurt and dairy products. The OMW was first clarified by using membrane technology and subsequently the contained poly-phenols were isolated by adsorption-desorption technique using selective macro-porous resins and finally recovered in dry form after been processed by RO membrane technique followed by freeze drying. Moreover, the polyphenols were encapsulated in modified starch by freeze drying in order to mask the color and bitterness effect and improve their functionality. The two products were used successfully as additives in yogurt preparations and the produced products were acceptable by the consumers and presented with certain advantage to the plain yogurt. For the herein proposed production scheme a patent application was already submitted.

**Keywords**—OMW, polyphenol-enriched yogurt, encapsulation, bio-active dairy products

## I. INTRODUCTION

SINCE 1991 when the “French paradox” was reported extensive studies were carried out in order to explore the functionality of the poly-phenols and their application in human health [1, 2]. As it is cited by Ruggeri et al. (2008) [3] there is an increasing interest by the consumer in foods that are recognized as beneficial for human health because they are either low fat or of greater nutritional value or contain bioactive materials which are associated with reduced risk of disease [4,5]. Ideally these foods should be appealing, taste good, be low in price and, most importantly for consumers’ acceptability, should contain all-natural ingredients, in a single, easily-accessible product. Fermented milk products, and in particular yogurts, also considered to be probiotic, represent a type of foods that attract greater interest with increasingly demanding and informed consumers [4]. New formulations of these food products are currently present in the food market, claiming different health benefits and presenting different physical and sensory characteristics—such as drinkable yogurts, probiotic yogurts, low-fat yogurts, and enriched yogurts—often offered to the consumer as original complex mixtures of microorganisms [6] designed to combine probiotic effects of bacteria with healthy positive effects of added substances such as polyphenols, coenzymes, or phytosterols.

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Several yogurt-based products are marketed with the addition of either fruit or vegetables rich in bioactive food ingredients or edible fibers claimed to have beneficial effects on human health.

The rationale behind these enrichments is that the ease of consumption of yogurt may improve body health status by maintaining a favorable intestinal microbial profile, possibly lowering cholesterol and blood pressure, and at the same time provide an optimal intake of bioactive components, often with beneficial antioxidant and free radical scavenging capacities [7]. This policy matches the high expectations of consumers and in turn encourages the consumption of fermented dairy products.

As it is reported by Sun-Waterhouse *et al* [1] polyphenols can be added to yogurt by pre- or post-fermentation approach, and this can potentially lead to: a) reactions with food components, e.g., interactions between milk proteins/polysaccharides and polyphenols [8,9,10,11,12,13], and/or b) interactions between polyphenols and starter cultures [14,15].

However, despite the fact that the polyphenols and other bioactive phytochemicals provide some distinctive positive health effects, some problems related to bad or bitter product taste or discoloration are encountered, especially with polyphenols, when they are added in some foodstuffs. The utilization of encapsulated polyphenols instead of free compounds can overcome the drawbacks of their instability, alleviate unpleasant tastes or flavors, as well as improve the bioavailability and half-life of the compound in vivo and in vitro [16]. In a recent comprehensive review by Fang & Bhandari [16] a series of encapsulation techniques are summarized concerning encapsulation of various natural polyphenols by using various techniques like: a) spray drying [17,18], b) coacervation [19,20] c) liposome inclusion [21,22] d) inclusion encapsulation [23,24] e) co-crystallization [25] f) freeze drying [26,27] g) nanoencapsulation [28,29] h) encapsulation in yeast cells [30] and i) emulsion encapsulation [31,32].

The aim of the present work was dual: to develop a method for production of plain (non-encapsulated) and starch-encapsulated OMW polyphenols and to investigate the potential of production of bioactive traditional Greek-type and European-type yogurt from sheep milk enriched with polyphenols in both plain and encapsulated forms.

## II. MATERIALS AND METHODS

### A. OMWW

1000 Kg of fresh OMW was collected from the olive mill of D. TSAKIRIDIS & Associates Co., located in Pournari, Larissa and stored at 0-4 °C in stainless steel tank to be used as raw material for polyphenol production.

### B. OMWW clarification and concentration

The OMWW was collected and stored in small batches of 200 Kg and each batch was treated with ceramic membrane microfiltration using a module equipped with the CMF19040 – 0.2 µm pore diameter (purchased by Jiangsu Jiuyu Hi-Tech Co. Ltd, 20 Xingke 3rd Road, Nanjing Hitech Development Zone, Jiangsu 210061, China). The membrane surface used was 0.23 m<sup>2</sup>. The obtained average flux value was greater than 20 Kg/m<sup>2</sup>hr, thus supporting economical industrial application. The concentration of the clarified OMWW was subsequently carried out by using a Reverse Osmosis (RO) technique in tubular mode configuration by using the PCI UK B1 tubular module equipped with 18 membrane tubes of PCI AFC99 with 99% rejection as NaCl and total membrane surface 0,86 m<sup>2</sup>. The concentration ratio used was 4:1 and the observed average flux was over 25 Kg/m<sup>2</sup>hr, which guaranties the potential of an economical commercial application. The retentate of RO was a polyphenol enriched dark liquid which was used in order to recover the polyphenols by preparative chromatography in the subsequent step while the permeate was pure de-ionized water.

### C. Recovery of OMWW polyphenols from the collected RO retentate

The recovery of the poly-phenols of the RO retentate was performed by using the experimental rig presented in Fig.1 According to the experimental procedure, as initial step, approximately 100 Kg of RO retentate was circulated through a bed of 30 Kg XAD4 Amperlite commercial polyphenol-selective macroporous resin for 24 hours by using a peristaltic pump at a flow rate of 100 lit/min. After that about 30 lt of water/ethanol solution (50:50 v/v) was used as eluting medium for about 3-4 hours in order to remove the adsorbed polyphenol from the resin. At the end of the elution step the alcoholic polyphenol solution was collected and concentrated by RO using the same scheme as for clarified OMW. The concentration ratio used was 5:1 and the RO flux was over 20 Kg/m<sup>2</sup> hr (commercial magnitude). The concentrated alcoholic polyphenol solution was subsequently processed by freeze drying by using the Scanvac Cool safe 110-4, Lab Freeze dryer (LaboGene, Denmark) to obtain a dark brown and bitter dry powder consisted of OMW polyphenols. Picture shows the recovered dry OMW powder before milling,

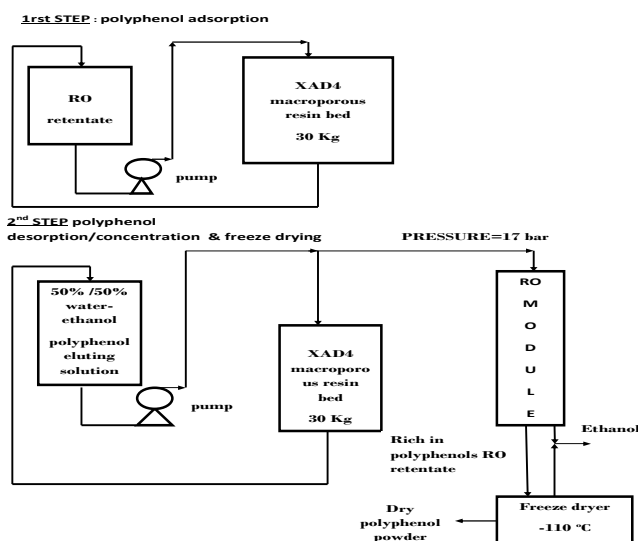


Fig. 1 The polyphenol recovery process

### D. Encapsulation of dry polyphenol powder in modified starch matrix

The produced dry OMWW polyphenol powder was encapsulated in modified starch matrix by using again the Scanvac Cool safe 110-4, Lab Freeze dryer (LaboGene, Denmark). The procedure used to obtain the encapsulated product was the following: 20g of plain dry polyphenol powder and 80 g thermo-resistant modified starch were diluted in 800 ml de-ionized water and the mixture was first homogenized and then were placed at -18 °C for 30 min in order to form a gel. This gel was then re-homogenized once more and distributed in the glass jars of the freeze dryer. The freeze drying cycle was completed at approx. 36 hrs and the morphology of the produced powder appears in Picture 2. It shows the morphology of the produced encapsulated polyphenol powder.



As shown in Picture 2 the encapsulated product had undergone an effective color masking. Also, the taste of the encapsulated product was not bitter any more, the product texture was very fine, thus implying very small particles, potentially due to the very sharp freezing by the operation of the freeze dryer at -110 °C.

### E. Membrane cleaning regime

The membrane cleaning regime was consisted of the following steps :

- 1) Rinsing with de-ionized water for 15 minutes
- 2) Filling of the stock tank with 50 Kg of hot water at 48 °C
- 3) Addition of Ultrasil 69 (360 g), Ultrasil 67(160 g), Ultrasil 02 (40 g) one after another with simultaneous stirring and circulation for 45 min.
- 4) Rinsing with hot water at 48 °C.
- 5) Filling of the stock tank with 50 Kg of hot water at 48 °C
- 6) Addition of acidic cleaner Ultrasil 75 (120 g) with simultaneous stirring and circulation for 15 min.
- 7) Rinsing with hot water at 48 °C
- 8) Filling of the stock tank with 50 Kg of hot water at 48 °C
- 9) Addition of alkali cleaner Ultrasil 110 (240 g) with simultaneous stirring and circulation for 15 min
- 10) Rinsing with hot water at 48 °C
- 11) Membrane preservation with 0.1% sodium bisulphate solution to prevent microbial growth.

All the cleaner solutions used were purchased by HENKEL-ECOLAB Greece.

### F. The method of yogurt production

30 Kg of sheep milk was first pasteurized at 90 °C for 6 minutes and then homogenized at 65 °C and 160 atm. The milk was brought at 46 °C and split into three equal weight portions of 10 Kg each. In portion A 500 ppm plain dry polyphenol powder was added, while in portion B 500 ppm encapsulated polyphenol powder was used, while in portion C no polyphenols were added. In all three portions the appropriate dose of the HANSEN YCX-11 yogurt starter culture was added. The culture was added at 41 °C. Each batch (A,B,C) was then filled in plastic containers of about 200 g and brought to thermal incubation at 42-44 °C in order for the appropriate fermentation and acidification to take place.

### G. DSC test of encapsulation efficiency

Differential Scanning Calorimetry (DSC) was used in order to ascertain and confirm the effectiveness of the encapsulation of polyphenol powder in modified starch. A DSC Q200 V 24.4. build 116 instrument (purchased by T&A Instruments, USA) was used in order to obtain the DSC graphs for plain polyphenol powder, modified starch and the encapsulated polyphenol powder respectively. The observed shift of the endothermic peak in the encapsulated derivative was used as criterion for effective encapsulation. The test was performed in ramp mode at 10 °C/min.

## III. RESULT AND DISCUSSION

### A. Comparison of the produced yogurt samples with and without poly phenol addition (Case1: traditional type Greek yogurt without aluminum cover).

TABLE I summarizes the comparative results for the three traditional Greek yogurt batches A (with plain polyphenols at

500 ppm addition, B (with encapsulated polyphenols 500 ppm) and C (without addition of polyphenols) concerning the reduction of pH value of the yogurt vs time and the microbiological count of the predominant spoilage micro-flora, namely yeasts and moulds.

TABLE I  
 PH VALUE AND YEAST AND MOULD COUNT OF THE PRODUCED TRADITIONAL GREEK TYPE YOGURT DURING FERMENTATION WITH AND WITHOUT POLYPHENOLS

	Test A (Addition of 500 ppm plain polyphenols)	Test B (Addition of 500 ppm encapsulated polyphenols)	Test C (Control-No addition of polyphenols)
Initial pH value	6,50	6,50	6,50
pH value after 2 hrs	6,48	6,40	6,40
pH value after 3 hrs	6,20	6,10	6,30
pH value after 4 hrs	4,80	4,70	5,40
At 24 hrs	pH 4,50	pH 4,55	pH 4,50
	<50 cfu/g pH 4,30	<50 cfu/g pH 4,28	<50 cfu/g pH 4,19
At 10 days	<50 cfu/g pH 4,25	<50 cfu/g pH 4,26	<100 cfu/g pH 4,10
At 15 days	<50 cfu/g	<50 cfu/g	<100 cfu/g
At 24 days	pH 4,23 <100 cfu/g	pH 4,21 <100 cfu/g	pH 4,08 >2000 cfu/g (mould appeared)

It can easily be observed from TABLE I that there is a substantial effect of the presence of the polyphenols in yogurt which can be summarized as follows: initially there is a lag time where the starter culture is not affected from the polyphenols while at a second stage after 2-3 hours the pH value of samples A, B containing the plain and encapsulated polyphenols respectively decreases faster than the pH value of sample C without polyphenols. This offers better protection against microbiological contamination and spoilage for the A and B samples. Furthermore, after the yogurt samples set in the fridge (0-4 °C), the polyphenol enriched yogurt samples A, B had a better self life while the control was spoiled by moulds in less than 24 days of storage. These findings support the hypothesis that the presence of plain or encapsulated polyphenols in yogurt provides protection for the product, initially due to a faster pH drop and later by decelerating mould development in the product.

This trend is presented graphically in Fig. 2 where the pH value of the three yogurt samples A, B and C is represented vs time for up to 24 days of storage.

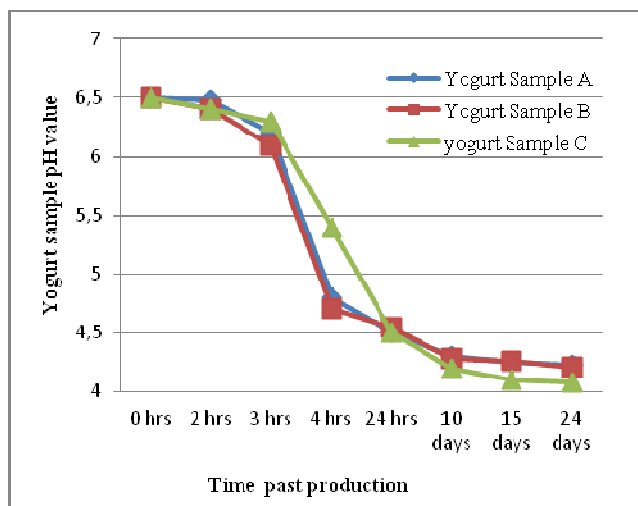


Fig. 2 The pattern of pH drop vs time for yogurt samples A, B, C

The rapid drop of the pH value of samples A, B (which contain the OMWW polyphenols) is also beneficial to yogurt production as the residence time of yogurt in the incubator at 41-42 °C is determined by the pH value which has to reach the value of 4.60-4.65. As seen in Fig. 1 this value is reached in the polyphenol enriched samples A and B in just 4 hrs while for sample C approximately 24 hrs are required. This obviously means a significant reduction in production time and at the same time energy savings for the yogurt industry.

*B. Comparison of the produced yogurt samples with and without polyphenol addition (Case II: European type yogurt with aluminum cover).*

In a second trial the produced yogurt was filled in plastic containers which were tightly closed by aluminum foil in order to be isolated from environmental contamination and thus to extend the expiry date. Two respective batches of this type of yogurt were produced: one with 1000 ppm encapsulated polyphenol addition and another with no addition of polyphenol.

TABLE II summarizes the comparative results for the two yogurt batches A (with encapsulated polyphenols at 1000 ppm addition, and B (without polyphenol addition), in relation to the reduction of pH value of the yogurt vs time and the total count of yeasts and moulds.

In this case, the presence of encapsulated polyphenols in yogurt at 1000 ppm produced a substantial acceleration of yogurt pH drop in sample A compared with sample B (without addition of poly-phenols). Furthermore, during the preservation of yogurt in chilled conditions (0-4 °C) the added encapsulated poly-phenols seem to excrete a protective effect against post-acidification of yogurt (during storage).

TABLE II  
 PH VALUE AND YEAST AND MOULD COUNT OF THE PRODUCED EUROPEAN TYPE YOGURT WITH AND WITHOUT POLYPHENOLS

	Test A	Test B
	(addition of 1000 ppm encapsulated)	(Control - No addition of poly phenols)

	polyphenols)	
Initial pH value	6,55	6,55
2 hrs	6,36	6,50
3 hrs	6,01	6,40
4 hrs	5,02	5,80
5 hrs	4,60	4,77
At 24 hrs	pH 4,51	pH 4,47
At 15 days	<50 cfu/g pH 4,48	<50 cfu/g pH 4,40
At 25 days	<50 cfu/g pH 4,44	<50 cfu/g pH 4,36
At 35 days	<50 cfu/g pH 4,42	<50 cfu/g pH 4,25
At 45 days	<50 cfu/g pH 4,40	<50cfu/g pH 4,20
	<50 cfu/g	<50cfu/g

In Fig. 3 the above mentioned trend is represented graphically

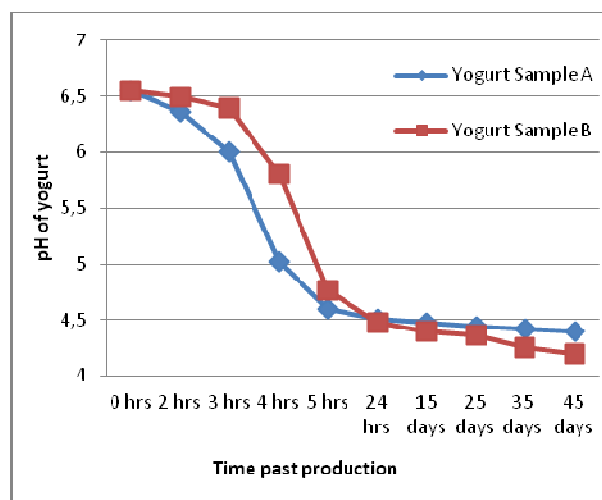


Fig. 3 The pattern of pH drop vs time for Yogurt samples A, B

By observing the data in Fig. 3 it is concluded that sample A which was spiked with 1000 ppm of encapsulated polyphenols exhibited a fast drop of pH value in the incubator and reached the critical pH value of 4.6 faster than the sample B without polyphenols, thus implying a benefit in energy costs and in production time for the polyphenol enriched yogurt. At the same time, in the long term, during storage of the product in chilled conditions (0-4 °C) the polyphenols seem to exert a protective effect against post-acidification of the yogurt and stabilize the pH value approximately at 4.4 while the same value for non polyphenol-enriched yogurt (Sample B) approaches a value of 4.2 at 25 day, which is not organoleptically acceptable by the consumer. This means that the presence of the polyphenols can extend the shelf life of the product by more than 10 days.

*C. Differential Scanning Calorimetry measurements for checking the encapsulation of poly-phenols*

The success of the encapsulation of the polyphenols was checked by Differential Scanning Calorimetry. Particularly, a

powder of plain polyphenol, modified starch powder (encapsulant) and the potentially encapsulated powder produced by homogenization and freeze drying were tested in the DSC apparatus and three DSC thermographs were produced which are presented in Fig. 4,5,6.

By comparing the three thermographs which all depict an endo-thermic peak, it can be deduced that the observed peak for the plain polyphenol powder is at 87.5 °C, for the starch powder approximately at 106 °C, while for the encapsulated polyphenol powder the peak has been moved at approx. 96 °C. This is a clear indication that a new type of particles has been formed with the polyphenol encapsulated into starch structure. At the same time the color of the encapsulated product appeared to be very light and its taste was not sharp bitter any more (as is the plain polyphenol powder), which are additional proofs for the successful encapsulation of the OMWW polyphenol in starch matrix.

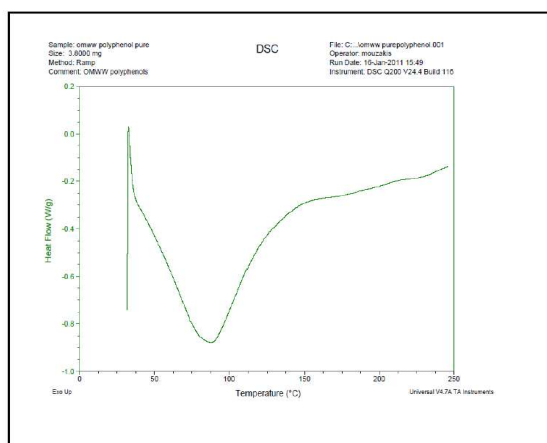


Fig. 4 The DSC thermo-graph of plain polyphenol powder

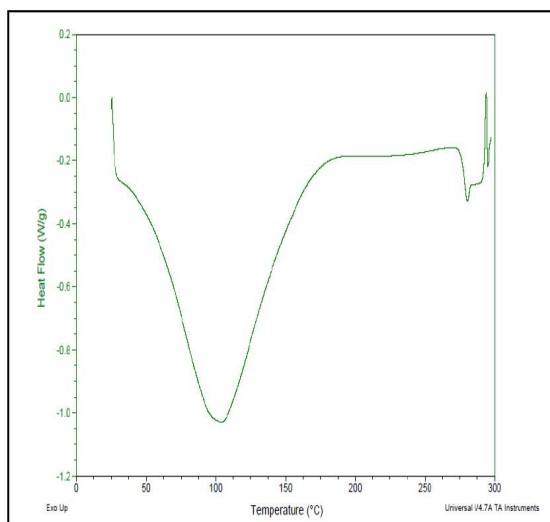


Fig. 5 The DSC thermo-graph of plain starch powder

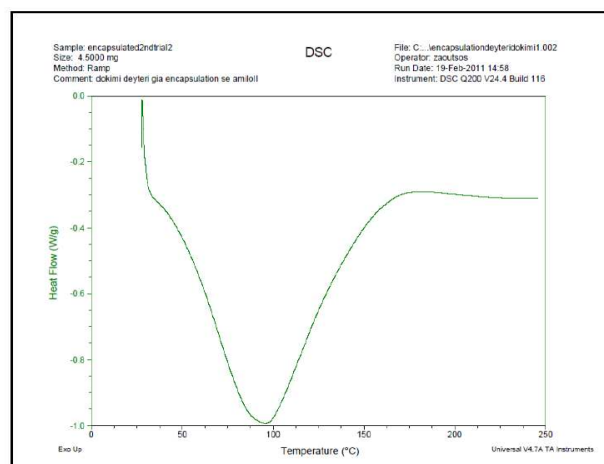


Fig. 6 The DSC thermo-graph of encapsulated polyphenol powder

#### D. The taste and appearance of the polyphenol enriched yogurt products.

The addition of plain polyphenol powder in the yogurt is not traced at all for the first days after the production. However, about two weeks from the production day the product presented the first signs of brown color probably due to the agglomeration of the polyphenols. This effect was completely absent when encapsulated polyphenol powder instead of plain polyphenol was added in the yogurt. This observation makes the use of encapsulated product more preferable due to lack of discoloration. In all cases, however, there was no sign of bitter taste in all polyphenol-enriched formulations tested.

#### IV. CONCLUSIONS

Olive mill wastewater (OMWW) polyphenols were successfully recovered using membrane technology to clarify and concentrate the OMWW in combination with preparative chromatography with macro-porous resin (adsorptive-desorptive technique) in order to recover polyphenols in 50/50 % ethanol/water solution which was then concentrated and plain polyphenol powder was produced (Product A) from the concentrate by freeze drying. The plain polyphenol powder was consequently encapsulated in thermo-resistant modified starch (Product B) and the encapsulation integrity was confirmed by DSC analysis. The encapsulated product contained 20% w/w polyphenol. Both the produced products A, B were incorporated in two yogurt preparations one traditional Greek yogurt not tightly sealed and a second European type filled in tightly sealed plastic containers. In all cases the pH value of the yogurt in the initial incubation step dropped substantially faster when this was enriched by polyphenols, and additionally the added poly-phenols (500-1000 ppm in concentration) exerted a protective effect against undesirable pH drop during yogurt storage (known as post-acidification). These two positive effects come as an addition to the enrichment of the yogurt with physical antioxidants coming from the olive fruit that have a positive effect to consumer health. For the new bioactive yogurt products enriched with OMWW polyphenols, a patent application is



pending by the authors, covering the novel scheme of polyphenol production and the OMWW polyphenol-enriched products themselves.

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