**Fast-fermented dry sausage without chemical additives/preservatives enriched with natural extracts of antioxidant and antimicrobial olive polyphenols**


## Brief Presentation of the Product

This is a research and development project conducted at the Technological Educational Institute (TEI) of Thessaly (formerly TEI of Larissa), in co-operation with the companies Tsianavas and Polyhealth S.A.
The product is a novel fast-fermented sausage of "clean label" (without chemical additives/preservatives) containing pork meat and fat, salt, pepper, garlic and natural extracts of olive polyphenols which is an excellent antioxidant and health-promoting food supplement.
Olive polyphenols (encapsulated in maltodextrin) derive from olive fruits by physical treatments only.

## The product's benefits and novel features include:

- The absence of ascorbate, which was substituted by olive polyphenols offering protection from lipid and color oxidation
- The absence of nitrites/nitrates (preservatives). Food safety and prolonged self life are ensured via the antimicrobial action of olive polyphenols (against certain pathogens/spoilage microorganisms), the protective culture used (which produces bacteriocins, organic acids, aromatic compounds) and the appropriate acidification and drying of the sausage
- The antifungal activity of olive polyphenols (at 2000ppm), which is evident by the absence of yeasts/moulds on the surface of the sausage casings
- The improved growth and acid production by the starter culture, which improve safety and can shorten the fermentation process.
- The presence of natural antioxidants from olive, which are very rich in hydroxytyrosol, a substance with significant antioxidant properties in food and the human body
- The presence of a very rich and distinct flavor, as a result of the addition of olive polyphenols

## What are olive polyphenols?

- Polyphenols, such as oleuropein, tyrosol, hydroxytyrosol, oleocanthal and oleacein found in olive, are extremely strong antioxidants.
- Their antioxidant activity is similar to artificial antioxidants like Butylatedhydroxyanisole(BHA) and Butylatedhydroxytoluene(BHT), and they are known to play multiple positive roles in our health.
- Polyphenols boost the immune system, protect us from heart diseases and display anticancer activity as they act as free radicals traps (scavengers).
- Several scientific publications underline the significant antioxidant, neuroprotective, antiaging, anticancer, and hypocholesterolaemic effects of olive polyphenols

- They protect olive oil from oxidative damage and they contribute to its superior oxidative stability among other edible oils.
- Hydroxytyrosol in particular, is a key antioxidant and antinflammatory substance found in olives, and aqueous olive extracts
- Recent data suggest that the health promoting properties of olive and olive oil, which are well known and exploited in food and medicine since ancient times, are mainly due to the polyphenolic content rather than the presence of monounsaturated fatty acids (MUFA)
- Interestingly, although known for their antimicrobial properties, recent data shows that olive polyphenols also seem to enhance growth and metabolism of lactic bacteria at certain levels applications in fermented food

**Production of dry fermented sausages -Our approach**

- We utilized the antioxidant and antimicrobial properties of the olive polyphenols that we isolated previously, in order to produce dry fermented sausages of high quality, clean label, with an additional nutritive/biological value
- To do this, we tested several concentrations and forms (free or encapsulated) of olive polyphenols, in order to find the optimal conditions for their incorporation into fermented sausages
- We omitted any kind of chemical additives or preservatives as we wanted to create a “healthy” sausage
- We used protective cultures of a mixture of lactic acid bacteria, staphylococci and yeasts which produce bacteriocins, organic acids and rich aroma, while it combats unwanted pathogens (e.g. Listeria monocytogenes).
- We achieved a faster and greater acidification of the product due to addition of olive polyphenols, which is crucial for the success of the fermentation and the safety of the product (similar results of induction of acid accumulation by lactic acid

bacteria have been shown in yogurt where olive polyphenol was added in order to accelerate acidification/coagulation)

- We ensured a low moisture content at the end of the fermentation (below 35%) which will not allow microbial spoilage or pathogen growth during chilled storage over several months.
- We formulated a novel product with competitive advantages, distinct character, innovative package, all at a reasonable price

**Production of dry fermented sausages with olive polyphenols– Recipe and Production Process**

- Frozen lean pork meat (from the laps and legs) and fat (from the hard parts of pork back) both of high quality and ≤ -6°C temperature are chopped and mixed in a cutter with salt, pepper, garlic, dextrose, the encapsulated polyphenolpowder and the starter culture (which also contains protective lactic acid bacteria).
- Raw materials in the initial product formulation:

•Pork meat 75%
•Pork fat 25%
•Salt 2,5%
•Dextrose 0,5%
•Pepper 0,3%
•Garlic 0,1%
•Encapsulated olive polyphenols2000ppm
•Bioprotective Starter Culture

The final product after a 3-week fermentation has lost ~35% of initial weight, thus its components have been condensed accordingly

***Critical control points (CCP) and limits during the consecutive production stages*:**
CCP1a. Microbiological criteria in Raw materials-Meat: Staphylococcus aureus<$10^2$cfu/g, Sulfite-reducing Clostridium spp. <$10^2$cfu/g, Enterobacteriacae<$10^2$cfu/g, Absence of Salmonella spp./25g, Absence of Listeriamonocytogenes/25g
CCP1b. Microbiological criteria in Raw materials-OMW polyphenols: Enterobacteriacae<$10^2$cfu/g, Sulfite-reducing Clostridium spp. <$10^2$cfu/g,
CCP1c. Microbiological criteria in Raw materials-Pepper and garlic: Sulfite-reducing Clostriumspp. <$10^2$cfu/g
CCP2. Temperature of raw meat and fat: (≤ -6°C)
CCP3. Temperature of processing unit:(≤ 12°C)
CCP4. Temperature in fermentation chamber: Maximum 24°C

***Critical control points (CCP) and limits during the consecutive production stages*:**
CCP5. Relative humidity in fermentation chamber: Maximum 95%, Minimum 75%
CCP6. pH of the final product: ≤5,3
CCP7. $a_w$of the final product≤ 0,95
CCP8. Vacuum packaging in polyethelen/polypropylene films:check for the presence of air/oxygen inside the package (<5% oxygen is expected throughout storage)
CCP9. Storage: at temperatures ≤ 4°C
CCP10. Microbiological criteria in the final fermented product (until the end of shelf-life):Staphylococcus aureus<$10^2$cfu/g, Sulfite-reducing Clostridium spp. <$10^2$cfu/g, Enterobacteriacae<$10^2$cfu/g, Absence of Salmonella/25g, Absence of L. monocytogenes/25g

The table below shows an average composition of the dry fermented sausages that we developed after a 3-week fermentation period

| **Component** | **Per100g serving** |
|---|---|
| Calories | 440 |

| | |
|---|---|
| Calories from fat | 325 |
| Fat (total) | 36g |
| Fat (saturated) | 12g |
| Carbohydrates | 1g |
| Protein | 27g |
| Sodium | 2,5g |
| Olive polyphenols | 0,3g |

**Production of dry fermented sausages with olive polyphenols-**
**Chemical/Physicochemical/Microbiological analyses**

- The microbiological analysis of the final product conformed with all the microbiological criteria as described above (in HACCP plan).
- Briefly, a significant reduction/inhibition of Clostridium spp. and Listeria monocytogenes(potential pathogens), as well as Pseudomonas spp. and Brochothrixspp. (potential spoilage organisms) was observed after addition of encapsulated olive polyphenols at 2000ppm, while protective cultures producing lactic acid and bacteriocins grew better and produced more acid in the presence of polyphenols at 2000ppm (compared to control samples without polyphenols).
- In terms of chemical and sensory analysis, resistance to oxidation of color and lipids (rancidity) was more effective by using encapsulated olive polyphenols than using ascorbate and nitrites.
- The photo below shows color formation after 3 days of fermentation in samples (from left to right) with ascorbates only, addition of encapsulated olive polyphenols at 250ppm, 500ppm, 1000ppm and 2000ppm, and samples with ascorbates and nitrites. The effect of olive polyphenols(2000ppm) was obvious.

- In preliminary tests with sausages containing ascorbates only (control), nitrites and ascorbates, or liquid condensed polyphenols(at 500 and 1000ppm), or polyphenols encapsulated in maltodextrin(at 200 and 4000ppm) we imposed a thermal and oxidation stress (4-week storage at 28°C under direct sunlight & air)
- Under these stress condition the protective effect of 400ppm encapsulated polyphenols was profound, keeping a bright red color and very low rancidity (tested organoleptically and as TBARs values), while the addition of ascorbates and nitrites was unable to preserve the bright red color and prevent an extended rancidity (see photo below).
- This experiment established the superiority of encapsulated polyphenols over the condensed liquid product of pure polyphenols, and thus only encapsulated polyphenol was used thereafter.


In the final tests we tested the microbiological, physicochemical and sensory attributes of fermented sausages with encapsulated polyphenols at 500, 1000, 2000ppm and 4000ppm, compared to a control containing nitrites and ascorbates.

- At 2000 and 4000ppm an antifungal effect was observed on the surface of the casing during fermentation, which prevented yeast/mold growth (see photo on the left). The photo on the left shows the comparative color and appearance of sausages on 17th day of fermentation with encapsulated polyphenols or nitrites-ascorbates. From left to right: sausage with nitrites-ascorbates, 500, 1000, 2000, 4000ppm encapsulated polyphenols

Since day 3 of the fermentation, it became apparent that polyphenols are substantial to color development in the absence of nitrites. The photo below shows from left to right the appearance and color of sliced sausages with ascorbates only (control), 250ppm, 500ppm, 1000ppm, 2000pm polyphenols and a combination of nitrites-ascorbates

The photo below shows the comparative color and appearance of sausages on 17th day of fermentation with encapsulated polyhenols or nitrites-ascorbates. From left to right: sausage

with nitrites-ascorbates, 500ppm, 1000ppm, 2000ppm, 40000ppm polyphenols.


In terms of chemical/sensory analysis, at 2000 (and 4000) ppm we observed a reduction in rancidity measured as TBAR's, and a faster rate of pH reduction and acid accumulation by lactic acid bacteria.

- Sensory attributes were optimal with 2000ppm polyphenols

In terms of microbiological quality and safety the products containing polyphenols conformed with all the microbiological criteria set in the HACCP Critical control points.

- At 2000 (and 4000) ppm we observed an antimicrobial effect against Clostridia and Pseudomonads, which is important for food safety and quality. On the contrary, growth of lactic acid bacteria was enhanced. Enterobacteriacae were within the set safety levels at the end of all fermentations
- **Important:**when we inoculated with 2,3 cfu/g and 46 cfu/g Clostridium perfringenson the first day of fermentation, we observed no growth during fermentation and in both cases the final product had <10cfu/g.

# Production of dry fermented sausages with olive polyphenols-final packing

## Indicative publications on the properties of olive polyphenols


1.JustinoCI,PereiraR,FreitasAC,Rocha-SantosTA,PanteleitchoukTS,DuarteAC.Oliveoilmillwastewatersbeforeandaftertreatment:acriticalreviewfromtheecotoxicologicalpointofview.Ecotoxicology.2012Mar;21(2):615-29.doi:10.1007/s10646-011-0806-y.Epub2011Nov1.

2.**Giavasis,I.,**E.Tsantel,P.Goutsidis,K.Papatheodorou,and**K.Petrotos**."Stimulatoryeffectofnovelpolyphenol-basedsupplementsfromolivemillwasteonthegrowthandacidproductionoflacticacidbacteria."MicrobesinAppliedResearch:CurrentAdvancesandChallenges(2012):308.

3.**KonstantinosB.Petrotos**,FaniK.Karkanta,PaschalisE.Gkoutsidis,**IoannisGiavasis**,KonstantinosN.Papatheodorou,AlexandrosC.Ntontos.ProductionofNovelBioactiveYogurtEnrichedwithOliveFruitPolyphenols.WorldAcademyofScience,EngineeringandTechnology,64,2012.

4.ElisaTripoli,MarcoGiammanco,GardenTabacchi,DanilaDiMajo,SantoGiammancoandMaurizioLaGuardia.Thephenoliccompoundsofoliveoil:structure,biologicalactivityandbeneficialeffectsonhumanhealth.NutritionResearchReviews,Volume18,Issue01,June2005,pp98-112.

5.FrancescoVisioli,AndreaPoli,ClaudioGall.Antioxidantandotherbiologicalactivitiesofphenolsfromolivesandoliveoil.MedicinalResearchReviewsVolume22,Issue1,pages65–75,January2002.

6.YvonneO′Dowd,FathiDriss,PhamMy-ChanDang,CaroleElbim,Marie-AnneGougerot-Pocidalo,CatherinePasquier,JamelEl-Benna.Antioxidanteffectofhydroxytyrosol,apolyphenolfromoliveoil:scavengingofhydrogenperoxidebutnotsuperoxideanionproducedbyhumanneutrophils.BiochemicalPharmacologyVolume68,Issue10,15November2004,Pages2003–2008.

7.Detaileddescriptionofolivepolyphenols,production,compositionandpropertiesbeneficialtohumanhealth(**ByPolyhealthS.A**.):http://www.polyhealth.gr/literature-about-products.