

## Effect of Some Inorganic Ions and Some Household and Industrial Wastes on The Biomass's Weight and Protein That Growth on Olive Mill Wastewater Medium

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### Abstract

The effect of some inorganic ions (nitrogen & phosphatase), dried domestic food residues (Fig fruit residues, Bean husks, Pea husks, Pomel husks) and some industrial wastes (Molasses - Olive sludge (solid waste of olive presses) On the weight of biomass developing in olive oil wastewaster OMW1, which is encrusted with Geotricum threads and extended with water extracts by 75:25 (OMW1: Aqueous extract of residues at concentration 1%,4%,7%,10%).

Nitrogen compounds had a significant effect on the increase in the mass of developing fungi compared to the control (distilled water distillation) as blank 1.0334gr/300ml dry weight where The concentration of Peas, Beans and molasses were effective at concentration (10-7), 4% and 10% respectively, with the mass increasing to 1.5346gr/300ml dry weight of Peas, 2.342gr/ 300ml dry weight of Beans and 2.5893gr/300ml of molasses. The rest of the water extracts (Phosphate compounds - Pomela husks - Fig) showed no apparent effect while sludge had a disincensive to the growth of bio-mass. (2.41g wet mushroom weight is equivalent to 0.03g dry weight) the effect of these extracts on the protein content of the biomass, Nitrogen compounds, Peas and Beans husks had a positive effect of 40% at 2.7% for Nitrogen and 30% at concentration greater than 7% for Peas and 25-20% at concentration greater than 4% For the bean extract, while the rest of the extracts (Phosphates, Pomela, Figs and Sludge) did not have any obvious effect.

**Keywords:** Olive mill wastewater – OMW

**Introduction:**

Olive cultivation is considered one of the most important crops in Syria. According to data from the Olive Office (Syrian Ministry of Agriculture 2010), Syria occupies the fourth place in the world in terms of number of trees and area planted with olives. Olive oil is extracted from olive fruits in a variety of ways, which has evolved over time between a mechanic that adopts traditional methods of pressing, or Three-phase centrifugation system or two-phase centrifugation system [1-9]. The percentage of oil produced by olive presses is 20% of the resulting quantity and 30% of solid waste and 50% of the product is in the form of liquid waste called Olive Mill Wastewaters (OMW) Of the remains of the internal olive pulp and a quantity of oil in addition to the washing water [6]. (OMW) is one of the topics that occupy researchers thinking for many reasons and from different points of view; it contains large proportions of water in places where water is scarce as a source of drinking and irrigation. Therefore, trying to find ways to use this water is an area to be dealt with. From another point of view, the disposal of large quantities of water residues containing pollutants contaminated with soil and groundwater is a matter of concern, except that the collections of these water releases foul gases and odors and constitutes a breeding ground for insects. OMW has several organic compounds, the most important of which are phenols, (simple phenols, and complex phenolsas piktins and Tannins ), They give this water its distinctive brown color, and this color tends to darken whenever it contains high concentrations of phenolic compounds. Phenols and their derivatives are bacterial growth inhibitors and have a detrimental effect on plant growth, making them unsuitable for agricultural use with high concentrations. OMW also contains fatty substances, glycosylated compounds, and proteinurics [6].

The problem of disposing of olive mill wastewater (OMW) remains unresolved, [5]. Some of the methods used to treat water residues from their chemical components (as compounds harmful to the environment) have focused on chemical methods and physical-chemical methods. By filtering the water with magnetic nanoparticles to remove pigmentation, remove sulfur and reduce the value of the oxygen chemical requirement [10] or by using micro filtration technology [11] or by chemical oxidation [3]. Many researchers have also used this liquid waste as a modus operandi to feed non-feed organisms such as the development of fungi and yeasts and the development of microorganisms consumed by organic compounds by disassembling

them and calculating the COD [7], Some researchers have also identified the treatment of olive oil by biodegradation by tricosporone kutaneum and giotrichocum [6], while others have shown that OMW can be used as a feeding medium for the development of certain species Fungi rich in proteins, carbohydrates and fats that may be suitable for use as feed additives[9],as Biological treatment of the olive waste of the olive press using bacteria [1].

### **Materials and preparation solution and samples:**

#### **1. Collection and preparation samples:**

Olive water samples: Samples from the water accompanying the OMW olive process were collected from a local contemporary (Syria) area close to Damascus countryside (Adiliya), where the most recent samples were selected (collected immediately after the press). Water samples of the olive mil waste water were incubated by heating them to inhibit enzymes from the microorganisms without removing the pods and later called OMW1 [9]. The samples were placed in sealed plastic containers and placed in refrigerators to maintain them at low temperatures for fear of side reactions that could occur between their components[13].

Samples of domestic plant waste: Pea, pea, Pomelo and fig fruits were collected from household waste after peeling and dried with electric manure at 50C ° and then grinded with electric grinder and vibrating sieve.

Beans: A plant species of legumes. His scientific name (in Latin: *Vicia faba*).

Peas: A plant species of the legume called *Pisum sativum*.

Pomegranate: A plant species that follows the species of citrus from the Siberian tribe called Sindi or Pomelo. The fruit is thick green crust.

Fig: A plant species of the Moraceae species called Latin *Ficus* .

**Preparation of the bio-material:** Olive fruits were washed, and the knife was washed, then immersed in water and left in the room temperature for a week [9]. And then took the threads of Haifa Figure (1) to do microscopic study to references, then it turned out to be Geotrichum threads Geotrichum [2], The last one was placed in a 300 mL tin container with a depth of 5 cm as in Fig. 2 for seven days at 28 ° C and in a medium containing:

1.6 g / monohydrogenate phosphatosphate, 0.025 g/l calcium chloride, 0.5g/l ammonium nitrate, 0.4 g/l mono-potassium phosphate, 0.2 g/l magnesium sulphate, seven water molecules and 0.0025 g/l Iron Chloride and 10 g/l



### **3- Experience methods**

The biomass was developed in medium of extension 75%:25% (OMW: aquatic extracts) in 300 mL round plastic containers with a depth of 3 cm for seven days at 28C° in the dark with continuous ventilation based on Previous experiments were identified in the study of this research and added distilled water every two days to compensate for what is missing[5] ,Then studied the amount of the resulting biomass and the amount of protein in it and compared with the growing biomass on OMW1 without any additions according to the following experiments:

**3.1 Calculation of the weight** of the living matter in the weight calculation method: The living matter is collected on the feeding medium and placed on a pre-weighted filter paper and then dried with the electric tank at 40C °. In calculating the difference between the two weights, the dry matter weight can be determined.

**3.2 Calculation of Total Nitrogen in Determination of Proteins** [12] The method of calcification in protein determination, which is based on the principle of conversion of ammonia nitrogen (in the studied organic samples) to ammonium sulphate by concentrated sulfuric acid, was followed by emancipation of ammonium by sodium hydroxyl 40% The water solution of the ammonia is reacted with the water chlorine acid and the HCl surplus is then adjusted with the sodium hydroxyl known as the concentration. After determining the total nitrogen content, the output is multiplied by 6.25 protein factors.

### **Results and discussion**

The effect of some of the inorganic and some of the dried household food residues (fig leaves, bean husks, pea husks, pomelo husks) and some of the industrial waste (molasses - olive sludge (solid residues of olive presses) The nutrient content of 300 mL of the olive oil extract was concentrated at a concentration of 75% + 25% of the water extracts of these residues at concentrations of 1%, 4% and 7%. 10% where the extract was prepared .

Effect of some inorganic acids (nitrogen and phosphate) on the weight of living matter developing on OMW1 and its protein content:

**1. Effect of nitrogen compounds:**

Nitrogen compounds such as potassium nitrate, ammonium nitrate and urea were studied with concentrations of 1%, 4%, 7% and 10%.

**1.1 Ammonium nitrate  $NH_4NO_3$**

**Table 1: biomass weight growth on OMW1 and protein ratio with different concentrations of  $NH_4NO_3$**

| Concentrate of $NH_4NO_3$  | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|----------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b> | 1.0334±0.1 <sup>a</sup> | 1.453±0.12 <sup>a</sup> | 2.076±0.12 <sup>c</sup> | 2.342±0.4 <sup>c</sup>  | 2.066±.2 <sup>c</sup>   |
| <b>Protein %</b>           | 13.64±0.27 <sup>a</sup> | 22.85±0.35 <sup>b</sup> | 31.67±0.43 <sup>c</sup> | 32.53±0.12 <sup>d</sup> | 32.54±0.25 <sup>d</sup> |

**2.1 Potassium nitrate**

**Table 2: biomass weight on OMW1 and protein ratio with different concentrations of  $KNO_3$**

| Concentrate of $KNO_3$     | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|----------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b> | 1.0334±0.1 <sup>a</sup> | 1.677±0.2 <sup>b</sup>  | 2.752±0.13 <sup>c</sup> | 2.742±0.1 <sup>c</sup>  | 2.298±0.2 <sup>d</sup>  |
| <b>Protein %</b>           | 13.64±0.27 <sup>a</sup> | 23.112±0.3 <sup>b</sup> | 38.9±0.23 <sup>c</sup>  | 40.12±0.16 <sup>d</sup> | 44.723±0.5 <sup>e</sup> |

**3.1 Urea  $CH_4N_2O$**

**Table (3) biomass weight on OMW1 and its protein ratio with different concentrations of  $CH_4N_2O$**

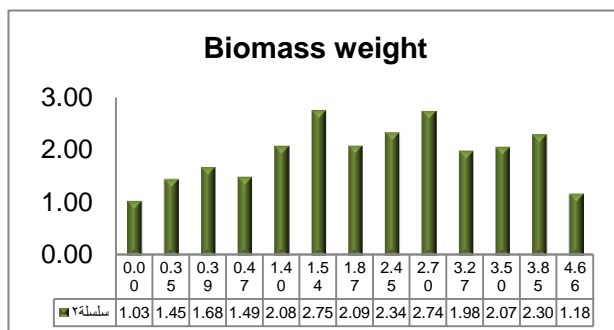
| Concentrate of $CH_4N_2O$  | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|----------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b> | 1.0334±0.1 <sup>a</sup> | 1.493±0.2 <sup>b</sup>  | 2.086±0.1 <sup>c</sup>  | 1.982±0.14 <sup>c</sup> | 1.175±0.23 <sup>a</sup> |
| <b>Protein %</b>           | 13.64±0.27 <sup>a</sup> | 25.170±0.4 <sup>b</sup> | 38.91±0.34 <sup>c</sup> | 38.75±0.41 <sup>c</sup> | 36.4±0.32 <sup>d</sup>  |

Table (4) shows the relationship between the protein content of the living biomass on OMW1 with different concentration of nitrogen oxides in the middle and the relationship between the biomass of the living material on the water residue of the olive press and the concentration of the nitrogen electrolytes. Comparison between the three tables (1), (2) 3).

**Table (4) Effect of nitrogen concentration on the biomass weight growth on OMW1 and its protein content**

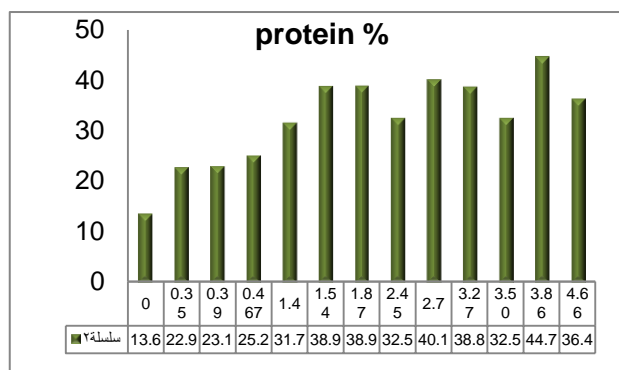
| Concentration of nitrogen 100 g / ml | Biomass weight | Protein content % |
|--------------------------------------|----------------|-------------------|
| 0                                    | 1.0334         | 13.64             |
| 0.35                                 | 1.4530         | 22.85             |
| 0.39                                 | 1.6773         | 23.11             |
| 0.467                                | 1.493          | 25.17             |
| 1.4                                  | 2.0768         | 31.67             |
| 1.54                                 | 2.7529         | 38.90             |
| 1.87                                 | 2.0866         | 38.91             |
| 2.45                                 | 2.3422         | 32.54             |
| 2.7                                  | 2.742          | 40.12             |
| 3.27                                 | 1.982          | 38.75             |
| 3.5                                  | 2.0665         | 32.545            |
| 3.86                                 | 2.298          | 44.723            |
| 4.66                                 | 1.1752         | 36.4              |

We conclude from the above that the presence of nitrogen in the oxidized form (potassium nitrate) or in reference form in the middle of growth (urea) increases the mass of biomass developing on OMW1, but the concentration of nitrogen is 2.7% in most of the growth media Figure (3)



**Figure (3)** highest concentration of nitrogen is 3.86%. As shown in Figure (4), in comparison with the three compounds, in the case of potassium the nitrate associated with nitrate is a more active stimulant for the protein synthesis than the ammonium residue.

In general, the appropriate concentration of nitrogen to increase the mass of living material is (1.4-3.86%) where at the higher



**Figure (4)** concentrations decreased the mass of living material began to decline.

## 2. Effect of phosphate compounds:

The biomass was developed on the water residue of the olive press (OMW1) after it was infused with geotricum threads in the center of the extension.

25:75 Water olive presses: Water additives for phosphate compounds (mono-potassium phosphate and double-potassium derivatives) at concentrations of 1%, 4%, 7%, 10% Determine the weight of the resulting living material and the amount of protein.

### 2.1 Mono-Potassium Phosphate $\text{KH}_2(\text{PO}_4)$

**Table (5)** The biomass weight that grew on OMW1 and its protein content with different concentration of  $\text{KH}_2(\text{PO}_4)_3$

| Concentrate of $\text{KH}_2(\text{PO}_4)_3$ | 0%                       | 1%                      | 4%                      | 7%                      | 10%                     |
|---|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Biomass weight (gr)                         | 1.4845±0.27 <sup>a</sup> | 1.521±0.34 <sup>a</sup> | 0.9497±0.5 <sup>a</sup> | 1.101±0.3 <sup>a</sup>  | 1.0334±0.1 <sup>a</sup> |
| Protein %                                   | 13.87±0.4 <sup>a</sup>   | 13.88±0.18 <sup>a</sup> | 13.37±0.2 <sup>a</sup>  | 13.50±0.25 <sup>a</sup> | 13.64±0.27 <sup>a</sup> |

### 2.2 Di-potassium Phosphate $\text{K}_2\text{H}(\text{PO}_4)_3$

**Table (6)** The biomass weight that growth on OMW1 and its protein content with different concentrations of  $\text{K}_2\text{H}(\text{PO}_4)_3$

| Concentrate of $\text{K}_2\text{H}(\text{PO}_4)_3$ | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|--|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Biomass weight (gr)                                | 1.4609±0.5 <sup>a</sup> | 1.249±0.42 <sup>a</sup> | 1.064±0.34 <sup>a</sup> | 1.023±0.25 <sup>a</sup> | 1.0334±0.1 <sup>a</sup> |
| Protein %  | 14.5±0.36 <sup>b</sup>  | 13.45±0.26 <sup>a</sup> | 13.37±0.12 <sup>a</sup> | 13.63±0.34 <sup>a</sup> | 13.64±0.27 <sup>a</sup> |

The figures (5) and (6) shows that there is no significant effect on the phosphorus density in all concentrations, either on the increase of the developing mass or on its protein content. The mass of living matter without



additives is 1.0334 g. We found that the proportion of protein did not exceed 14.5% with the presence of phosphorus reagent, noting that the proportion of protein in the biomass without any additives is 13.64%.

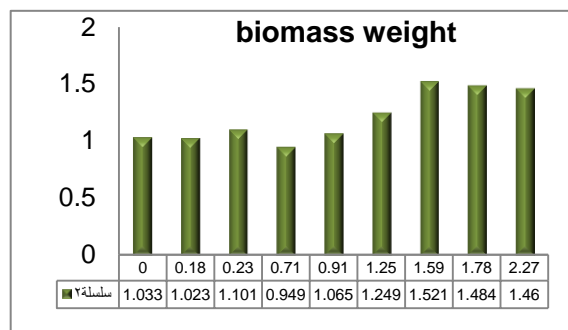


Figure (5)

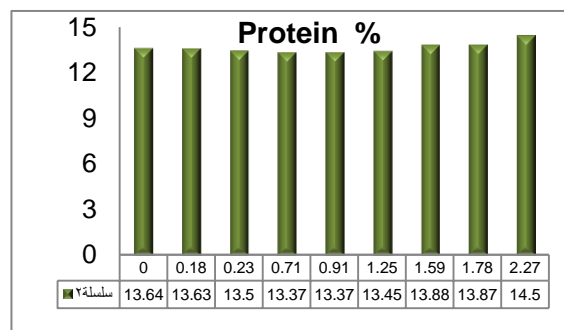


Figure (6)

Table (7) also shows the effect of phosphorus concentration in OMW1 on the weight of biomass and its protein content.

| Concentration of Phosphor 100 g / ml | Biomass weight | Protein content % |
|--------------------------------------|----------------|-------------------|
| 0                                    | 1.0334         | 13.64             |
| 0.18                                 | 1.023          | 13.63             |
| 0.23                                 | 1.101          | 13.50             |
| 0.71                                 | 0.949          | 13.37             |
| 0.91                                 | 1.0648         | 13.37             |
| 1.25                                 | 1.249          | 13.45             |
| 1.59                                 | 1.521          | 13.88             |
| 1.78                                 | 1.484          | 13.87             |
| 2.27                                 | 1.460          | 14.5              |

### 3. The effect of some household waste on the weight of living matter developing on OMW1 and its protein content

The effect of some of the dried domestic food residues (fig leaves, bean husks, pea husks, pea husks) on the mass of the living material and its protein content on the water residue of the olive press OMW1 and coated with the geotrichum thread within 7 days was measured at a total size of 300 ml in the center of the extension 75% 25% of the water extracts of these residues with concentrations of 1%, 4%, 7%, and 10%.

#### 3.1 Effect of fig fruit residues:

The effect of fig fruit was found to inhibit biomass growth. The growth rate was almost 40%, but did not affect protein content. Table 8 showed that protein content increased by 33% compared to the control (0%).

**Table (8) The biomass weight on OMW1 and the protein content in different concentration of fig fruit**

| concentration of solution extract fig fruit | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|---|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b>                  | 1.0334±0.1 <sup>a</sup> | 1.002±0.32 <sup>a</sup> | 0.781±0.45 <sup>a</sup> | 0.823±0.32 <sup>a</sup> | 0.655±0.27 <sup>a</sup> |
| <b>Protein %</b>                            | 13.64±0.27 <sup>a</sup> | 14.41±0.26 <sup>a</sup> | 15.54±0.42 <sup>b</sup> | 18.21±0.5 <sup>c</sup>  | 16.53±0.3 <sup>d</sup>  |

### 3.2 Effect of bean husk residues

Table (9) shows that the water extract of bean husk increased the mass of the living matter by 100% at the concentration of 7% and its protein content compared with the control (0%).

**Table (9) The biomass weight on OMW1 and its protein content with different concentration of bean husks**

| concentration of solution extract bean husks | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|--|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b>                   | 1.0334±0.1 <sup>a</sup> | 0.987±0.23 <sup>a</sup> | 1.2705±0.4 <sup>a</sup> | 2.342±0.5 <sup>b</sup>  | 2.2608±0.5 <sup>b</sup> |
| <b>Protein %</b>                             | 13.64±0.27 <sup>a</sup> | 18.96±0.43 <sup>b</sup> | 21.93±0.14 <sup>c</sup> | 25.85±0.23 <sup>d</sup> | 24.82±0.37 <sup>e</sup> |

### 3.3 Effect of pea husk residues

Table (10) shows that the water extract of pea scales increases the mass of living matter by 50%, but protein content increased by more than 100% compared to the control (0%).

**Table (10) The biomass's weight on OMW1 and biomass's its protein content with different concentration of pea husks**

| concentration of solution extract pea husks | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|---|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b>                  | 1.0334±0.1 <sup>a</sup> | 0.879±0.18 <sup>a</sup> | 0.942±0.43 <sup>a</sup> | 1.173±0.32 <sup>a</sup> | 1.534±0.45 <sup>a</sup> |
| <b>Protein %</b>                            | 13.64±0.27 <sup>a</sup> | 17.32±0.5 <sup>b</sup>  | 23.075±0.4 <sup>c</sup> | 29.9±0.23 <sup>d</sup>  | 31.35±0.34 <sup>e</sup> |

### 3.4 Effect of Pomela husk residues

It was found that there is no effect of the water extract of the bovine crusts in both cases either on the growth of the biomass or on its protein content. As Table 11 shows, the protein content increased by a maximum of 13% compared to the control (0%).

Table (11) The biomass weight on OMW1 and its protein ratio with different concentration of the pomelian husks

| concentration of solution extract pomelian husks | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|--|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b>                       | 1.0334±0.1 <sup>a</sup> | 0.944±0.14 <sup>a</sup> | 0.728±0.19 <sup>a</sup> | 0.934±0.32 <sup>a</sup> | 1.067±0.3 <sup>a</sup>  |
| <b>Protein %</b>                                 | 13.64±0.27 <sup>a</sup> | 13.34±0.21 <sup>a</sup> | 12.93±0.34 <sup>a</sup> | 13.65±0.5 <sup>a</sup>  | 15.52±0.17 <sup>b</sup> |

#### **4. Effect of some industrial wastes on the weight of the living biomass on OMW1 and its protein content**

##### **4.1 Effect of molasses:**

Table (12) shows that molasses have the effect of increasing the mass of developing living matter but have no apparent effect on protein content

Table (12) The biomass weight on OMW1 and its protein content with different concentration of molasses

| concentration of molasses extract solution | 0%                      | 1%                      | 4%                      | 7%                      | 10%                     |
|--|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>Biomass weight (gr)</b>                 | 1.0334±0.1 <sup>a</sup> | 1.156±0.31 <sup>a</sup> | 1.709±0.22 <sup>a</sup> | 2.274±0.47 <sup>b</sup> | 2.589±0.25 <sup>b</sup> |
| <b>Protein %</b>                           | 13.64±0.27 <sup>a</sup> | 14.12±0.33 <sup>a</sup> | 14.675±0.3 <sup>a</sup> | 17.47±0.19 <sup>b</sup> | 18.215±0.6 <sup>b</sup> |

##### **4.2 Sludge Effect:**

Table (13) shows that no sludge has any effect on protein content, but it has an inhibitory effect on growth of biomass on OMW1 surface.

Table (13) The biomass weight on OMW1 and its protein content with different concentration of sludge

| concentration of molasses extract solution | 0%                      | 1%                      | 4%                      | 7%                      | 10%                      |
|--|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|
| <b>Biomass weight (gr)</b>                 | 1.0334±0.1 <sup>a</sup> | 0.829±0.35 <sup>a</sup> | 0.4328±0.5 <sup>a</sup> | 0.562±0.45 <sup>a</sup> | 0.5169±0.36 <sup>a</sup> |
| <b>Protein %</b>                           | 13.64±0.27 <sup>a</sup> | 13.43±0.32 <sup>a</sup> | 13.95±0.45 <sup>a</sup> | 11.47±0.26 <sup>a</sup> | 12.52±0.43 <sup>a</sup>  |

#### **Conclusions**

The use of compounds containing nitrogen oxides and water extracts of bean and pea extracts can be used to develop biomass by adding them to a medium of olive oil as a 25% extension medium and to obtain a biomass containing a protein that can be studied subsequently as feed additives. An analytical study of the resulting proteins and their acid content Amino is also recommended to study the analysis of the biomass resulting in whether they

contain vitamins or carbohydrate or lipid material can be used in addition to proteins.

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