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# EFFECT OF SOME CHEMICAL AND BIOLOGICAL TREATMENTS ON THE CHEMICAL COMPOSITION, CELL WALL CONSTITUENTS AND *IN SITU* DEGRADABILITY OF OLIVE CAKE NUTRIENTS

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**ABSTRACT:** The objective of this investigation is to evaluate impacts of some chemical and biological treatments on the chemical composition, cell wall constituents and in situ degradability of olive cake (OC). OC was treated by water (control, T<sub>1</sub>), 8% molasses (T<sub>2</sub>), 7% urea (T<sub>3</sub>), 8% molasses + 7% urea (T<sub>4</sub>), 8% molasses + 1.5% sodium hydroxide (T<sub>5</sub>), 8% molasses + 3% sodium hydroxide (T<sub>6</sub>), 8% molasses + 6% lime (T<sub>7</sub>), 8% molasses + 8% lime (T<sub>8</sub>), 8% molasses + 10% lime (T<sub>9</sub>), 8% molasses + 0.25% Cata pro $^{\otimes}$  (T<sub>10</sub>), 8% molasses + 0.5% Cata pro $^{\otimes}$  (T<sub>11</sub>) and 8% molasses + 1% Cata pro<sup>®</sup> (T<sub>12</sub>). The initial moisture in all treatments was adjusted at 65%. The obtained results revealed that the chemical and biological treatments of OC for 30 days had positive advances on the chemical composition of OC, where the crude protein values increased, while the crude fiber contents decreased. The best improvements were occurred by adding molasses with lime (T<sub>9</sub>) or with 1% Cata pro<sup>®</sup> (T<sub>12</sub>). Also, the neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and total tannins decreased with all treatments. T<sub>9</sub> recorded the least content of cellulose, however the lowest percentage of total tannins was observed with T<sub>6</sub>. Among the biological treatments of OC, the maximum reduction in cellulose content accompanied by the minimum concentration of total tannins was detected with T<sub>12</sub>. All treatments led to improve crude protein, disappearance of NDF, ADF and ADL after 24 and 48 hours of the in situ degradability of OC. The T<sub>9</sub> and T<sub>12</sub> were the most effective treatments. Conclusively, the best improvement of chemical composition, cell wall constituents and in situ degradability of OC were occurred by 8% molasses + 10% lime (T<sub>9</sub>), followed by 8% molasses + 1% Cata pro $^{\text{@}}$  (T<sub>12</sub>).

**Key words:** Olive cake, chemical and biological treatments, chemical composition, cell wall, *in situ* degradability.

### **INTRODUCTION**

In Egypt, the gap in requirements of the concentrate feeds reached nearly 4.3, 3.4 and 0.3 million tons as dry matter (DM), total digestible nutrients (TDN), and digestible crude protein (DCP), respectively (Shata and Ebrahem, 2014). Therefore, many researchers targeted the utilization of agro-industrial by-products to close the huge gap in animal feeds. The utilization of agro by-products as animal feed will shrink feeding costs, play a role of self-sufficiency and reduce the problem of environmental pollution (Ajila et al., 2012).

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In Egypt large quantities of OC is available. Where the production of olive fruits reached about 700 thousand tons (Ministry of Agriculture and Land Reclamation, 2015). Each ton of olive fruits generates about 0.55 to 0.80 ton of OC (Molina-Alcaide and Yáñez-Ruiz, 2008). Using of OC is limited due to several factors such as the low degradability of DM, crude protein (CP) and neutral detergent fiber (NDF), high quantity of the nitrogen content is attached to the cell wall, heating during the extracting of olive oil can form tannin-protein complexes, low nutritive value which attributed to it's high content of lignin and

low energy and digestible protein contents (Nefzaoui, 1999 and Yansari et al., 2007). Inhancing the nutritive values of OC by chemical (Rowghani et al., 2008; Ashraf et al., 2013; Ishfaq et al., 2017) and biological treatments (Obeidat, 2017 and Abd El-Tawab et al., 2018) were discussed. However, there are positive effects of enzyme addition on the animal production (Elghandouret al., 2015) and the relative feeding cost (Shaaban, 2016).

The present work was designed to investigate the effect of some chemical and biological treatments on the chemical composition, cell wall constituents and *in situ* degradability of OC nutrients.

### **MATERIALS AND METHODS**

This work was conducted at Animal Production Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt and South Sinai research station, Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt, during the period from 2016 till 2018.

#### **Treatments of Olive Cake**

Olive cake was obtained from a private olive oil extraction press mill, Ras Sudr, South Sinai Governorate, Egypt. The chemical treatments included addition of urea, sodium hydroxide or calcium hydroxide (lime). The chemical additives were purchased from El Gomhouria Company for Drugs, Chemicals and Medical Supplies, branch of Zagazig District, Egypt. While, the biological treatments involved addition of Cata pro<sup>®</sup> (a commercial product). Cata pro<sup>®</sup> produced by Catalysis S.L, Madrid, Spain. Each kg of Cata pro® contains: Aspergillus oryzae extracts (1250 units of xylanase, 275 units of hemicellulase, 225 units of  $\beta$ -glucanase), 50 g of Bacillus subtilis extract (2500 units of αamylase, 450 units of cellulase, 12500 units of protease,10000 mg betaine HCl 98%, 10 g Lactobacillus acidophilus (200 million CFU/g), 5 g Enterococcus faecium, 48 g Lactobacillus blantarum, 0.2 g Bifido bacterium bifidum and complemented to 1000 g using dextrose as a diluent. All additives (chemical or biological) were supplemented on dry matter basis (DM), dissolved in water and then sprayed on OC. The initial moisture was adjusted to 65%. Thereafter, the treatments of OC were mixed thoroughly,

backed in plastic jars and tightly closed. OC treated by water (control,  $T_1$ ), 8% molasses ( $T_2$ ), 7% urea ( $T_3$ ), 8% molasses + 7% urea ( $T_4$ ), 8% molasses + 1.5% sodium hydroxide ( $T_5$ ), 8% molasses + 3% sodium hydroxide ( $T_6$ ), 8% molasses + 6% lime ( $T_7$ ), 8% molasses + 8% lime ( $T_8$ ), 8% molasses + 10% lime ( $T_9$ ), 8% molasses + 0.25% Cata pro® ( $T_{10}$ ), 8% molasses + 0.5% Cata pro® ( $T_{11}$ ) and 8% molasses + 1% Cata pro® ( $T_{12}$ ). Representative samples were taken from each treatment and stored at -20 °C till chemical analysis. Then, proximate chemical analysis, cell wall constituents (CWC) and *in situ* evaluation were performed.

### In situ Ruminal Degradability

The polyester bag technique was used to measure the in situ of DM disappearance (ISDMD), crude protein disappearance (ISCPD), NDF disappearance (ISNDFD), ADF disappearance (ISADFD) and ADL disappearance (ISADLD) of OC (Ørskov et al., 1980). Samples were dried and ground by a grinder with a 2 mm sieve. Five g of each sample was transferred into polyester bags (12  $\times$  6 cm) with 50  $\mu$ m pore size. Four bags per each of inculcation time (24 and 48 hours) were subjected to the ruminal degradability by using three fistulated Barki rams (average weight 55 kg) fed on berseem hay and concentrates at 1 and 2% of live weight, respectively. After each incubation time, bags were removed and washed with cold running tap water until the water remained clear. The bags were oven dried at 60°C for 48 hours, then weighted.

#### **Statistical Analysis**

Data of *in situ* experiment were subjected to analysis of variance (SAS, 2004). Significant differences between treatment means were tested by Duncan's Multiple Range Test (Duncan, 1955).

### RESULTS AND DISCUSSION

# Effect of Chemical and Biological Treatments on Chemical Composition and pH Value of OC

The chemical composition and pH values of OC are presented in Tables 1 and 2. All treatments led to decrease the organic matter (OM), crude fiber (CF) and increased crude protein (CP) contents in compared to olive cake (OC) control. The pH values increased by all treatments, except that with molasses alone  $(T_2)$ .

Table 1. Effect of treatments on chemical composition (on fresh basis) and pH values of olive cake

Item	Chemical composition (%)						pН		
Treatment	Moisture	DM	OM	EE	CF	CP	NFE	Ash	
Raw OC	26.15	73.85	70.05	12.34	28.64	7.79	21.28	3.80	6.20
$T_1$ (control)	35.75	64.25	61.02	10.63	26.09	5.38	18.92	3.23	5.20
T <sub>2</sub> (8% molasses)	35.50	64.50	60.78	10.38	25.55	6.32	18.52	3.72	4.97
T <sub>3</sub> (7% urea)	39.77	60.23	57.51	9.55	22.47	16.50	8.99	2.72	9.00
T <sub>4</sub> (8% molasses+ 7% urea)	36.93	63.07	59.15	9.74	19.92	18.16	11.33	3.92	8.84
T <sub>5</sub> (8% molasses+ 1.5% sodium hydroxide)	39.58	60.42	56.47	9.67	22.38	5.81	18.61	3.95	7.01
$T_6$ (8% molasses+ 3% sodium hydroxide)	35.25	64.75	59.39	9.74	21.39	6.59	21.67	5.36	7.51
$T_7(8\%molasses + 6\%calciumhydroxide)$	40.45	59.55	52.38	8.87	16.92	5.65	20.94	7.17	6.50
$T_8$ (8% molasses+ 8% calcium hydroxide)	40.30	59.70	51.85	8.37	16.97	6.56	19.95	7.85	7.00
T <sub>9</sub> (8% molasses+ 10% calcium hydroxide)	36.97	63.01	54.19	8.47	17.54	7.17	21.01	8.82	6.81
$T_{10}$ (8% molasses+ 0.25% cata pro®)	41.96	58.04	54.06	9.74	19.04	5.92	19.36	3.98	5.60
$T_{11}$ (8% molasses+ 0.5% cata pro®)	40.20	59.80	55.84	10.61	19.23	5.65	20.34	3.96	5.72
$T_{12}$ (8% molasses+ 1% cata pro®)	41.01	58.99	55.25	10.52	17.87	5.80	21.07	3.74	5.41

The moisture adjusted to be 65% ( $T_1$  to  $T_{12}$ ). EE: Ether extract, NFE: Nitrogen free extract

Table 2. Effect of treatments on chemical composition (on dry matter basis) and pH values of olive cake

Item	Chemical composition (%)						
Treatment	DM	OM	EE	CF	CP	NFE	Ash
Raw OC	100	94.85	16.71	38.78	10.55	28.81	5.15
$T_1$ (control)	100	94.98	16.55	40.61	8.38	29.44	5.02
T <sub>2</sub> (8% molasses)	100	94.23	16.10	39.62	9.80	28.71	5.77
T <sub>3</sub> (7% urea)	100	94.48	15.85	37.30	27.40	13.93	5.52
T <sub>4</sub> (8% molasses+ 7% urea)	100	93.79	15.45	31.58	28.80	17.96	6.21
T <sub>5</sub> (8% molasses+ 1.5% sodium hydroxide)	100	93.46	16.01	37.04	9.61	30.8	6.54
T <sub>6</sub> (8% molasses+ 3% sodium hydroxide)	100	91.72	15.05	33.04	10.18	33.45	8.28
T <sub>7</sub> (8% molasses+ 6% calcium hydroxide)	100	87.96	14.9	28.77	9.48	34.81	12.04
$T_8$ (8% molasses+ 8% calcium hydroxide)	100	86.85	14.02	28.42	10.99	33.42	13.15
T <sub>9</sub> (8% molasses+10% calcium hydroxide)	100	86.00	13.45	27.83	11.38	33.34	14.00
T <sub>10</sub> (8% molasses+ 0.25% cata pro®)	100	93.14	16.78	32.80	10.20	33.36	6.86
T <sub>11</sub> (8% molasses+ 0.5% cata pro®)	100	93.37	17.74	32.16	9.45	34.02	6.63
$T_{12}$ (8% molasses+ 1% cata pro®)	100	93.66	17.83	30.29	9.82	35.72	6.34

Findings of adding molasses alone or urea treatments (with or without molasses) are consistent with those obtained by Rowghani and Zamiri (2007). They revealed that CP contents in all of OC silages including urea were significantly higher than those in other silages. Additionally, CP in OC which treated with molasses plus urea was enhanced compared to that treated with urea only. The same trend was found with Aboul-Fotouh et al. (2013) who referred to enhancement in CP content accompanied by a decrease in CF content for urea-treated OC. The high values of pH are due to the high level (7%) of urea addition. On the other hand, outcomes of sodium hydroxide treatments agreed with Abo Omar et al. (2012) concerning values of CP, CF and EE and with Nefzaoui and Vanbelle (1986) as regards to the pH values. Results of Ca (OH)2 treatments are in with Ashmawy accordance (2011)mentioned that CF and EE values were significantly decreased, while the contents of OM, NFE, and ash were significantly increased. Also, **Ishfaq** et al. (2015) reported that the contents of OM and CF were significantly lowered when OC treated with slaked lime (6%). They added that the content of CP was insignificantly increased, while marginally decreased. The findings of biological additives are in accordance with Fadel and El-Ghonemy (2015) who reported that the fungal treatment of OC by Aspergillus oryzae besides molasses addition resulted in a rise in CP accompanied with a decline in CF. Also, **Abdou** (2017) stated that enzymatic treatment of OC mixture led to increasing the CP, NFE and EE contents, while decreased the CF content in comparison with the untreated one. As regards to the pH-value results, there is an agreement with Abd-El Tawab et al. (2018) who revealed that the pH value with the enzyme-treated OC silage slightly differed in comparison with that in the untreated one.

# Effect of Chemical and Biological Treatments on Cell Wall Constituents and Total Tannins of OC

Contents of NDF, ADF, ADL and total tannins were decreased with all additives used compared to those of the raw OC and the control  $(T_1)$  as shown in Table 3. Contents of

hemicellulose, cellulose and lignin were lowered with  $T_9$  by about 58.1, 38.0 and 26.98%, respectively as relative values of their comparable contents in the control. On the other side,  $T_2$  led to lowering total tannins by about 18.89% in parallel with that of the control. Also, the total tannins were reduced considerably by 28.87% in  $T_6$  compared to the control. Additionally,  $T_9$  resulted in substantial reductions in total tannins contents approaching 20.38, respectively as relative to that of the control. Regarding the biological treatments, notable decreases in values of cellulose (27.27%) and total tannins (13.38%) with  $T_{12}$  in comparison with the control.

Effects of chemical treatments on cell wall constituents (CWC) are consistent with those referred by Abo Omar et al. (2012) who treated OC with NaOH (4%), Rowghani and Zamiri (2007) who ensiled OC with molasses (8%) or urea (0.5%) or both of them, and by **Ishfaq** et al. (2015) who treated OC with slaked lime (6%). On the other side, outcomes of biological treatments on CWC agreed with Neifar et al. (2013) who treated OC with Fomes fomentarius. Contents of NDF, ADF and ADL were significantly reduced with the fungal treated OC. As well, Fadel and El-Ghonemy (2015) cleared that fungal treatment of OC with Aspergillus oryzae decreased the NDF and ADF contents. Further, Abdou (2017) attributed the reduction of the CWC contents in OC to Allzyme® supplementation (0.5, 1.0 and 1.5%) through breakdown the linkage between lignin and the other cell wall components, then releasing it mainly the hemicellulose. However, the low values of total tannins in OC with all treatments are in line with Weinberg et al. (2008) who stated that nearly 40% of polyphenols was lowered during the ensiling process. In addition, Fadel and El-Ghonemy (2015) reported that the fungal treatment of OC with Aspergillus oryzae plus supplementing molasses (2.5%) reduced the content total phenols by about 78%. Concisely, the results mentioned above cleared that chemical and biological treatments of OC for 30 days had desired enhancements on the chemical composition of OC, especially with adding molasses plus lime or with Cata pro<sup>®</sup>.

Table 3. Effect of treatments on cell wall constituents and total tannins (%) of olive cake

Item	Neutral detergent fiber (NDF)	Acid detergent fiber (ADF)	Acid detergent lignin (ADL)	Hemicellulose	Cellulose	Lignin *	Total tannins
Treatment	A de fibe	Acid	Acid de lignin	Неп	ŭ	H	43
Raw OC	73.00	57.36	34.72	15.64	22.64	33.07	4.83
$T_1$ (control)	65.15	50.83	32.75	14.32	18.08	31.10	4.71
T <sub>2</sub> (8% molasses)	63.70	50.60	32.34	13.10	18.26	30.69	3.82
T <sub>3</sub> (7% urea)	61.95	49.69	31.30	12.26	18.39	29.65	4.48
T <sub>4</sub> (8% molasses+ 7% urea)	56.67	42.41	29.51	14.46	12.70	27.86	4.75
$T_5 \ (8\% \ molasses + 1.5\% \ sodium \ hydroxide)$	61.29	47.94	32.60	13.35	15.34	30.95	3.85
$T_6$ (8% molasses+ 3% sodium hydroxide)	54.80	42.87	29.50	11.93	13.37	27.85	3.35
T <sub>7</sub> (8% molasses+ 6% calcium hydroxide)	51.23	43.75	21.08	7.48	22.67	19.43	4.00
T <sub>8</sub> (8% molasses+ 8% calcium hydroxide)	45.38	33.49	20.86	11.89	12.63	19.21	3.42
$T_9 \ (8\% \ molasses + \ 10\% \ calcium \ hydroxide)$	41.57	35.57	24.36	6.00	11.21	22.71	3.75
$T_{10}~(8\%~molasses+~0.25\%~cata~pro\circledast)$	61.12	46.59	26.26	14.53	20.33	24.61	4.50
$T_{11}$ (8% molasses+ 0.5% cata pro®)	57.18	42.71	24.67	14.47	18.04	23.02	4.10
$T_{12}$ (8% molasses+ 1% cata pro®)	55.34	38.68	25.53	16.66	13.15	23.88	4.08

<sup>\*</sup>Lignin = ADL – [average (%) of acid insoluble ash in OC which was 1.65%].

### Effect of Chemical and Biological Treatments on ISDMD

The ISDMD values of the treated OC were significantly increased with all treatments compared to that with the control (T1, water alone), except that with  $T_2$  (molasses alone). The amount of amelioration was varied according to the type of treatment used (Table 4). After 24 and 48 hours of ruminal incubation, ISDMD values with T<sub>2</sub> did not significantly differ in comparison with  $T_1$ . With  $T_4$ , the ISDMD values were better (P<0.05) than those with  $T_3$ , after 24 and 48 hours of ruminal incubation. Increasing the level of NaOH from 1.5 to 3% led to enhance (P<0.05) the ISDMD values. In the same trend, values of ISDMD were significantly (P<0.05) increased by using ascending levels of lime. Among all treatments, T<sub>9</sub> recorded the best average ISDMD, where it ameliorated by 215 and 198% after 24 and 48 hours, respectively

compared to those with  $T_1$  (100%). All levels of Cata pro® supplementation, significantly (P<0.05) improved the ISDMD values. Using the level 1% of Cata pro® in  $T_{12}$  resulted in improvements of ISDMD by about 154 and 195% after 24 and 48 hours, respectively of *in situ* ruminal degradability as a proportion of that with  $T_1$  (100%).

Yansari et al. (2007) elucidated that the low ISDMD of OC is one of the essential limiting factors of using it in ruminant's feeding. The usage of OC with suitable supplements could be useful in animal nutrition. Our findings are in concurrence with Rowghani and Zamiri (2007), since the average ISDMD with supplementing urea plus molasses to OC was significantly higher than that with adding urea alone. Also, the results agreed with Nefzaoui and Vanbelle (1986) concerning the maximum in sacco digestibility of the ensiled OC was

Table 4. Effect of treatments on *In situ* dry matter disappearance (ISDMD) of olive cake

Item	Dry n disappe	Relative improvement (%)		
Treatment	24 hours			48 hours
$T_1$ (control)	$28.84^{e} \pm 0.75$	$35.24^{\text{fg}} \pm 0.42$	100	100
T <sub>2</sub> (8% molasses)	29.61 <sup>e</sup> ±0.68	$33.40^{\mathrm{g}} \pm 0.71$	102.67	94.78
T <sub>3</sub> (7% urea)	$27.73^{e} \pm 2.29$	$39.66^{e} \pm 2.57$	96.15	112.54
T <sub>4</sub> (8% molasses+ 7% urea)	$40.14^{cd} \pm 2.24$	$48.04^{\circ} \pm 1.39$	139.18	136.32
$T_5$ (8% molasses+ 1.5% sodium hydroxide)	$29.94^{e} \pm 0.20$	$38.19^{ef} \pm 0.66$	103.81	108.37
$T_6$ (8% molasses+ 3% sodium hydroxide)	$37.15^{d} \pm 2.38$	$43.52^{d} \pm 1.86$	128.81	123.50
T <sub>7</sub> (8% molasses+ 6% calcium hydroxide)	$52.87^{b}\pm2.13$	$59.07^{\ b} \pm 0.87$	183.32	167.62
$T_8$ (8% molasses+ 8% calcium hydroxide)	57.98 a ±1.23	$61.21^{b} \pm 1.39$	201.04	173.70
T <sub>9</sub> (8% molasses+ 10% calcium hydroxide)	62.09 a ±0.71	69.89 a ±0.71	215.30	198.32
$T_{10}$ (8% molasses+ 0.25% cata pro®)	$36.92^{d} \pm 0.90$	$50.71^{c} \pm 1.19$	128.02	143.90
T <sub>11</sub> (8% molasses+ 0.5% cata pro®)	$40.75^{cd} \pm 1.12$	$49.40^{\circ} \pm 0.65$	141.30	140.18
$T_{12}$ (8% molasses+ 1% cata pro®)	$44.27^{\circ} \pm 0.57$	$68.78^{a} \pm 1.17$	154.00	195.17

a, b, c, d, e, f and g are means in the same column with different superscripts are significantly different (P<0.05).

recorded with the highest concentration of NaOH. Similarly, the results are in the same line with **Ashmawy (2011)** who reported that values of ISDMD and ISOMD of OC were significantly superior with the highest level of lime (12%, for a month). Likewise, there was an agreement with **Fadel and El-Ghonemy (2015)** who revealed that biological treatment with *Aspergillus oryzae* could be an efficient organism for production of the lignocellulolytic enzymes and consequently enhancing the digestibility of OC.

### Effect of Chemical and Biological Treatments on ISCPD

There were significant (P<0,05) increase in the ISCPD values after 24 and 48 hours of incubation in rumen with all treatments compared to that of control (Table 5). Among all treatment of OC,  $T_4$  recorded the highest (P<0.05) ISCPD value after 24 hours of the ruminal incubation. In addition, the ISCPD values in  $T_4$  were higher (P<0.05) than those in  $T_3$  at both sampling times. Rising of the NaOH level from 1.5 to 3% led to an increase (P<0.05) in the ISCPD values. In the same manner, the

values of ISCPD were enhanced (P<0.05) with the highest level of lime ( $T_9$ ), where the relative improvement of ISCPD reached 141.32% after 48 hours of incubation in the rumen compared to  $T_1$  (100%). Also, the former trend was observed with  $T_{12}$  (1% Cata pro<sup>®</sup>), which resulted in the greatest (P<0.05) relative improvement of ISCPD value among all treatments at the second sampling time (141.65%) compared to that with  $T_1$  (100%).

Yansari et al. (2007) cleared that the low ISCPD of OC is one of the main limiting factors of using it in ruminant's feeding. The least degradable fraction in crude OC was the protein. A high quantity of the nitrogen in OC is attached to the cell wall. The heating during the extracting of olive oil can form tannin-protein complexes. The usage of OC with suitable supplements could be useful in animal nutrition. These results are in agreement with Rowghani and Zamiri (2007) and Rowghani et al. (2008) who revealed that the average ruminal ISCPD with the treatment supplemented by urea plus molasses was significantly better than the untreated OC or that with OC supplemented by urea alone. In addition, our results are in the

Table 5. Effect of treatments on *In situ* crude protein disappearance (ISCPD) of olive cake

Item		protein earance	Relative improvement (%)		
Treatment	24 hours	24 hours 48 hours		48 hours	
T <sub>1</sub> (control)	36.98 g ±3.71	64.52 h ±1.15	100	100	
T <sub>2</sub> (8% molasses)	$53.82^{e} \pm 1.28$	$74.69^{fg} \pm 1.12$	145.54	115.76	
T <sub>3</sub> (7% urea)	$77.52^{b} \pm 0.75$	$81.90^{\circ} \pm 0.53$	209.63	126.94	
T <sub>4</sub> (8% molasses+ 7% urea)	84.09 a ±0.63	$87.78^{b} \pm 0.71$	227.39	136.05	
T <sub>5</sub> (8% molasses+ 1.5% sodium hydroxide)	$63.54^{d} \pm 0.36$	$78.88^{ed} \pm 1.02$	171.82	122.26	
T <sub>6</sub> (8% molasses+ 3% sodium hydroxide)	$77.01^{b} \pm 1.28$	$82.94^{c} \pm 1.97$	208.25	128.55	
T <sub>7</sub> (8% molasses+ 6% calcium hydroxide)	$69.82^{\circ} \pm 0.19$	$80.61^{cd} \pm 0.42$	188.80	124.94	
$T_8$ (8% molasses+ 8% calcium hydroxide)	$70.12^{c} \pm 0.70$	$83.49^{\circ} \pm 0.92$	189.62	129.40	
T <sub>9</sub> (8% molasses+ 10% calcium hydroxide)	$74.73^{b} \pm 0.47$	91.18 a ±0.91	202.08	141.32	
$T_{10}$ (8% molasses+ 0.25% cata pro®)	$44.81~^{\rm f}\pm0.78$	$77.38^{ef} \pm 0.48$	121.17	119.93	
T <sub>11</sub> (8% molasses+ 0.5% cata pro®)	$50.03^{e} \pm 1.58$	$72.65^{\text{ g}} \pm 0.35$	135.29	112.60	
T <sub>12</sub> (8% molasses+ 1% cata pro®)	$62.33^{d} \pm 1.56$	91.39 a ±0.41	168.55	141.65	

a, b, c, d, e, f and g are means in the same column with different superscripts are significantly different (P<0.05).

same trend with **Nefzaoui** *et al.* (1982) as announced by **FAO** (1985) which published that the alkaline treatments (NaOH and NH<sub>3</sub>) of OC led to notable advances in the ruminal ISCPD values. They added that ISCPD values were increased with elevating the concentrations of the alkaline. Upgrading the ISCPD with Cata pro<sup>®</sup> (1%) may attributed to that the microbial protein synthesis in the rumen was enhanced with the biological supplementation as reported by **Van de Vyver and Useni**, (2012).

### Effect of Chemical and Biological Treatments on ISNDFD

The ISNDFD values of control did not significantly differ with  $T_2$ after 48 hours incubation in rumen (Table 6). Among all treatments, ISNDFD values with  $T_9$ was significantly (P<0.05) better than those with the other treatments. The increase of NaOH, Ca (OH)<sub>2</sub> or Cata pro® levels led to improve ISNDFD values. Where,  $T_9$  enhanced the ISNDFD values by about 211 and 227 % after 24 and 48 hours of the ruminal incubation, respectively in parallel with  $T_1$  (100%). Regarding the biological

additive,  $T_{12}$  achieved the best ISNDFD values. This level (1% of Cata pro<sup>®</sup>) ameliorated the ISNDFD values by about 139 and 172% after 24 and 48 hours, respectively of *in situ* ruminal degradability as a proportion of that with  $T_1$  (100%).

Yansari et al. (2007) mentioned that the low ISNDFD of OC is one of the major limiting factors of using it in ruminant's feeding. The usage of OC with suitable supplements could be useful in animal nutrition. As far as we know, scarce trials have investigated the effects of alkaline and biological treatments of OC on ISNDFD. Concerning lime treatments, Ashraf et al. (2013) mentioned that the improved digestibilties of lime treated OC (up to 8%) were attributed to that internal hydrogen bonding became fragile and then weaken the fiber structure in OC. Our results can be supported with similar results obtained by Abd El Tawab et al. (2018). They added the exogenous enzymes to OC before ensiling. They noticed that the best value of in vitro NDF disappearance (42.20%) was recorded with including the treated OC silage level at 75% in the diet.

Table 6. Effect of treatments on *In situ* neutral detergent fiber disappearance (ISNDFD) of olive cake

Item		detergent ppearance	Relative improvement (%)		
Treatment	24 hours	48 hours	24 hours	48 hours	
$\overline{T_1(control)}$	34.35 h ±0.17	36.79 g ±0.52	100	100	
$T_2(8\% \text{ molasses})$	$36.53^{fgh} \pm 1.05$	$38.36^{g} \pm 1.04$	106.35	104.27	
T <sub>3</sub> (7% urea)	$39.21^{ef} \pm 0.40$	$41.75~^{\rm f} \pm 0.20$	114.15	113.48	
T <sub>4</sub> (8% molasses+ 7% urea)	$44.91^{d} \pm 1.54$	$45.05^{ef} \pm 0.41$	130.74	122.45	
T <sub>5</sub> (8% molasses+ 1.5% sodium hydroxide)	$35.28^{gh} \pm 1.33$	$45.62^{de} \pm 0.92$	102.71	124.00	
$T_6$ (8% molasses+ 3% sodium hydroxide)	$44.43^{d} \pm 0.65$	$46.42^{de} \pm 1.01$	129.34	126.17	
T <sub>7</sub> (8% molasses+ 6% calcium hydroxide)	$61.89^{b} \pm 0.42$	$66.20^{\circ} \pm 0.46$	180.17	179.94	
$T_8$ (8% molasses+ 8% calcium hydroxide)	$62.53^{\text{ b}} \pm 0.73$	$71.23^{b} \pm 1.41$	182.04	193.61	
T <sub>9</sub> (8% molasses+ 10% calcium hydroxide)	$72.60^{a} \pm 0.82$	$83.39^{a} \pm 1.73$	211.35	226.66	
$T_{10}$ (8% molasses+ 0.25% cata pro®)	$37.48^{fg} \pm 0.40$	$41.84^{\rm \ f} \pm 0.97$	109.11	113.73	
$T_{11}$ (8% molasses+ 0.5% cata pro®)	$40.73^{e} \pm 1.57$	$48.73^{d} \pm 2.39$	118.57	132.45	
$T_{12}$ (8% molasses+ 1% cata pro®)	$47.66^{\circ} \pm 0.85$	$63.13^{\circ} \pm 0.41$	138.75	171.59	

a, b, c, d, e, f, g and h are means in the same column with different superscripts are significantly different (P<0.05).

## Effect of Chemical and Biological Treatments on ISADFD

The ISADFD values of the treated OC were significantly improved with all additives, except that with T<sub>2</sub> after 48 hours of incubation compared to that with  $T_1$  (Table 7). In all treatments, the average ISADFD was improved after 48 hours in parallel with those recorded after 24 hours of ruminal incubation. The extent of betterment was differed according to the type of the used treatment. With T4, the ISADFD values were battered (P<0.05) in parallel with those T<sub>3</sub> at both sampling times. Rising levels of NaOH and Ca(OH)<sub>2</sub> resulted in increasing the ISADFD values. At the first sampling time, T<sub>9</sub>recorded the greatest average ISDMD, where it increased by 205 % compared to T<sub>1</sub> (100%). At the second sampling time (48 hours),  $T_{12}$ recorded the best (P<0.05) values of ISADFD among all treatments. This level of Cata pro® (1%) led to improve the average ISADFD by about 225% after 48 hours of in situ ruminal degradability as relative to that with  $T_1$  (100%).

Our findings are in line with Alvarez-Rodríguez et al. (2009) who indicated that the value of ISADFD in the crude OC was progressed after 72 hours of ruminal incubation. A notable advance in the average ISADFD of the treated OC with graded concentrations of NaOH (4 to 8%) which was better than those with the same concentrations of ammonia (Nefzaoui et al., 1982) as announced by FAO (1985). Also, Nefzaoui and Vanbelle (1986) revealed that the ensiled OC with ascending levels of ammonium hydroxide (4, 6 and 8% on dry basis) or with the same levels of NaOH achieved significant enhancements of ISADFD values compared to those of the untreated one. The strongest effect of NaOH treatment on the ISADFD value OC detected with the highest level of it. As regard to the results of Cata pro® supplementation, they can be confirmed with comparable results obtained by Abd El-Tawab et al. (2018). They added the exogenous enzymes to OC before the ensiling process. They noticed that the best values of in vitro ADF disappearance (30.98%) were observed when treated OC silage incorporated in the diet at the level of 75%.

Table 7. Effect of treatments on In situ acid detergent fiber disappearance (ISADFD) of olive cake

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Treatment Item	Acid dete disapp	Relative improvement (%)		
	24 hours	48 hours	24 hours	48 hours
T <sub>1</sub> (control)	$24.63^{\text{ f}} \pm 0.49$	30.13 h ±0.37	100	100
$T_2(8\% \text{ molasses})$	$29.12^{e} \pm 1.12$	$30.26^{h} \pm 0.76$	118.23	100.43
T <sub>3</sub> (7% urea)	$30.30^{\mathrm{e}} \pm 0.55$	$33.17^{\mathrm{g}} \pm 0.73$	123.02	110.09
T <sub>4</sub> (8% molasses+ 7% urea)	$33.90^{d} \pm 0.43$	$35.99^{\mathrm{f}} \pm 0.44$	137.64	119.45
$T_5$ (8% molasses+ 1.5% sodium hydroxide)	$28.60^{\mathrm{e}} \pm 0.36$	$37.01^{\mathrm{f}} \pm 0.88$	116.12	122.83
T <sub>6</sub> (8% molasses+ 3% sodium hydroxide)	$33.74^{d} \pm 0.46$	$40.01^{e} \pm 0.23$	136.99	132.79
T <sub>7</sub> (8% molasses+ 6% calcium hydroxide)	$43.23^{\rm c} \pm 0.82$	$45.47^{d} \pm 0.41$	175.52	150.91
T <sub>8</sub> (8% molasses+ 8% calcium hydroxide)	$46.35^{b} \pm 0.61$	$52.46^{\circ} \pm 0.69$	188.18	174.11
T <sub>9</sub> (8% molasses+ 10% calcium hydroxide)	$50.59^{a} \pm 0.77$	$62.46^{b} \pm 1.29$	205.40	207.30
$T_{10}~(8\%~molasses+~0.25\%~cata~pro\circledast)$	$29.89^{e} \pm 0.40$	$40.90^{e} \pm 1.19$	121.36	135.74
T <sub>11</sub> (8% molasses+ 0.5% cata pro®)	$30.51^{e} \pm 0.75$	$50.80^{\circ} \pm 0.58$	123.87	168.60
T <sub>12</sub> (8% molasses+ 1% cata pro®)	$29.96^{e} \pm 0.65$	$67.84^{a} \pm 1.58$	121.64	225.16

a, b, c, d, e, f, g and h are means in the same column with different superscripts are significantly different (P<0.05).

## Effect of the Chemical and Biological Treatments on ISADLD

In comparison with  $T_1$  (control),  $T_2$  slightly improved the ISADLD, while the average ISADLD values were bettered (P>0.05) with the other additives at both sampling times (Table 8). At the second sampling time, T<sub>4</sub> enhanced (P<0.05) the average ISADLD compared to that with T<sub>3</sub>. The average ISADLD was increased with the graded concentrations of NaOH, Ca(OH)<sub>2</sub> and Cata pro<sup>®</sup>. Among treatments, T<sub>9</sub> recorded the best ISADLD value at the first sampling time. Where, it raised by 197 % as a proportion of that with OC control (100%). While, the superior ISADLD value at the second sampling time was detected with T<sub>12</sub> which upgraded the average ISADLD nearly 244% as relative to that of control (100%).

Ashraf et al. (2013) mentioned that the superior digestibilities of alkaline treated OC were attributed to that internal hydrogen bonding became fragile and then weaken the fiber structure in OC. Concerning the biological additive (Cata pro®), Muwalla et al. (2007) stated that feed digestion may be improved with the addition of exogenous enzymes through hydrolyzing feed directly or work synergistically with ruminal microorganisms. As well as, there was a correspondence with results illustrated by Fadel and El-Ghonemy (2015) who reported that the lignocellulolytic enzymes of Aspergillus oryzae could be effective in enhancing the digestibility of OC.

### Conclusion

The best improvement in chemical composition, CWC and *in situ* degradability of OC were detected with  $T_9$  (8% molasses + 10% lime and  $T_{12}$  (OC + 8% molasses + 1% Cata pro<sup>®</sup>).

Table 8. Effect of treatments on *In situ* acid detergent lignin disappearance (ISADLD) of olive cake

Item	Acid det lignin disa <sub>l</sub>		ative ment (%)		
Treatment	24 hours 48 hour		24 hours	48 hours	
T <sub>1</sub> (control)	12.21 g ±0.54	15.55 h ±0.44	100	100	
T <sub>2</sub> (8% molasses)	$13.75^{\rm fg} \pm 0.62$	$15.84^{h} \pm 0.69$	112.61	101.86	
T <sub>3</sub> (7% urea)	$16.15^{\text{cde}} \pm 0.41$	$20.67^{\text{ g}} \pm 0.68$	132.27	132.93	
T <sub>4</sub> (8% molasses+ 7% urea)	$17.69^{\text{ c}} \pm 0.47$	$23.83^{fe} \pm 0.50$	144.88	153.25	
$T_5 \ (8\% \ molasses + 1.5\% \ sodium \ hydroxide)$	$15.35^{def} \pm 0.54$	$21.83^{\mathrm{g}} \pm 0.66$	125.72	140.38	
$T_6$ (8% molasses+ 3% sodium hydroxide)	$17.66^{\text{ c}} \pm 0.44$	$26.35^{d} \pm 0.44$	144.63	169.45	
T <sub>7</sub> (8% molasses+ 6% calcium hydroxide)	$20.71^{\ b} \pm 1.29$	$21.32^{\mathrm{g}} \pm 0.41$	169.61	137.11	
$T_8$ (8% molasses+ 8% calcium hydroxide)	$22.72^{a} \pm 0.44$	$25.15^{\text{ de}} \pm 0.49$	186.08	161.74	
T <sub>9</sub> (8% molasses+ 10% calcium hydroxide)	$24.10^{\ a}\pm0.47$	33.25 <sup>b</sup> ±0.62	197.38	213.83	
$T_{10} \ (8\% \ molasses + 0.25\% \ cata \ pro \circledast)$	$14.72^{ef} \pm 0.41$	$22.04^{fg} \pm 0.81$	120.56	141.74	
$T_{11}$ (8% molasses+ 0.5% cata pro®)	$16.87^{cd} \pm 0.76$	$29.02^{\circ} \pm 0.59$	138.16	186.62	
$T_{12}$ (8% molasses+ 1% cata pro®)	$17.50^{\circ} \pm 0.35$	37.89 a ±0.92	143.32	243.66	

a, b, c, d, e, f, g and h are means in the same column with different superscripts are significantly different (P<0.05).

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## تأثير بعض المعاملات الكيميائية والحيوية على التركيب الكيماوى ومكونات جدار الخلية واختفاء المركبات الغذائية لكسب الزيتون في الكرش

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أجريت هذه الدراسة لمعرفة تأثيرات بعض المعاملات الكيميائية والحيوية على التركيب الكيميائي، مكونات جدار الخلية، والهضم الموقعي في الكرش لكسب الزيتون، تم معاملة كسب الزيتون بالماء (كنترول م،) ،  $^{0}$  مو  $^{0}$   $^$ 

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