Determination of Effects of Microwave Irradiation on Fermentation of Oak Nut (*Quercus coccifera*) Using Hohenheim Gas Production Technique

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ABSTRACT: The aim of the current study was to determine the effects of drying with microwave irradiation on chemical composition, fermentation, metabolisable energy and organic matter digestibility of oak nut (*Quercus coccifera*) using Hohenheim *in vitro* gas production technique. Microwave irradiation was applied to oak nut for 0, 2, 4, 6 and 8 minutes. Microwave irradiation has no significant effect on the chemical composition except for dry matter and condensed tannin contents of oak nut. Microwave irradiation increased the dry matter contents whereas microwave irradiation decreased condensed tannins of oak nut. Although microwave irradiation has a significant effect on the gas production at early incubation times, it has no significant (P<0.001) effect on gas production at late incubation times. At early incubation times gas production linearly increased with time of microwave irradiation. Microwave irradiation has no effect on the estimated parameters such as the total gas production, metabolisable energy and organic matter digestibility whereas it increased gas production constant (c) of the plant. As a conclusion, microwave irradiation can be used to dry oak nut with high moisture when harvested without affecting the chemical composition and nutritive value of oak nut. However the effect of microwave irradiation should be tested further with *in vivo* experiment before large implication.

Key Words: Digestibility, gas production, metabolisable energy, microwave irradiation, oak nut,

Mikrodalga Isıl İşleminin Meşe Palamudunun Fermantasyonuna Etkisinin Hohenheim Gaz Üretim Tekniği Kullanılarak Belirlenmesi

ÖZET: Bu çalışmanın amacı mikrodalga ışınlama ile kurutmanın meşe palamudunun kompozisyonuna, fermentasyonuna, metabolik enerji ve organik madde sindirim derecesine etkisini belirlemektir. Meşe palamutları 0, 2, 4, 6 ve 8 dakika mikrodalga ışınlamaya tabi tutulmuştur. Mikrodalga ışınlama kuru madde ve kondense tanen hariç meşe palamutlarının kompozisyonunu etkilememiştir. Mikrodalga ışınlama kuru madde içeriğini artırırken kondense tanen içeriğini azaltmıştır. Mikrodalga ışınlama inkübasyonun erken saatlerinde gaz üretimini artırırken inkübasyonun ilerlemesiyle birlikte gaz üretiminde farklar görülmemiştir. İnkübasyonun erken saatlerinde gaz üretimin mikrodalga ışınlamaya bağlı olarak lineer bir artış göstermiştir. Mikrodalga ışınlamanın meşe palamudunun toplam gaz üretim miktarına, metabolik enerji ve organik madde sindirim derecesine önemli bir etkisi olmamıştır. Sonuç olarak meşe palamudunun kompozisyonunu ve besleme değerini etkilemeden, mikrodalga ışınlama yüksek nem içerikli meşe palamudunu kurutmak için kullanılabilir. Bununla birlikte geniş çaplı uygulamalardan önce mikrodalga ışınlamanın etkileri *in vivo* denemelerle test edilmelidir.

Anahtar Kelimeler: Sindirim derecesi, gaz üretimi, metabolik enerji, mikrodalga ışınlama, meşe palamudu,

INTRODUCTION

Recently there has been a growing interest in industrial application of microwaves to improve conventional drying processes since the application of microwaves has several advantages such as lower startup time, faster heating, greater energy efficiency, space savings, precise process control, selective heating and yielding final products with improved nutritive quality when compared with conventional drying processes. Microwaves irradiation results in gelatinization of grain, which led to chemical and physical changes in the starch granules and facilitated starch availability for micro-organisms to ferment them (Khajehdizaj et al. 2013). In addition, microwave irradiation decreased the tannin content of Mucuna pruriens var utilis bean (Kala and Mohan. 2011).

Recently several studies have been carried out to determine the effect of microwaves irradiation on the fermentation kinetics of grains and agro-industrial byproduct using *in vitro* gas production technique (Maheri-Sis et al. 2011a, Khajehdizaj et al. 2013, Parnian et al. 2013, Paya et al. 2014). However the effect of microwaves irradiation is not consistent. Microwave irradiation for 3, 5 and 7 min increased (P<0.05) gas production, organic matter digestibility, metabolisable energy, net energy lactation and short chain fatty acids content of sorghum whereas irradiation has no effect on these parameters of wheat grain (Parnian et al. 2013).

The fruit of oak trees is a nut called an acorn which has similar content to that of cereal grains. The attempts in microwaves irradiation of cereal grains encouraged to

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test the idea of microwaves irradiation of oak acorn with high moisture. So far there is no attempt to microwaves irradiation of the acorn of oak trees in the literature. Therefore the aim of the current experiment was to determine the effects of drying with microwave irradiation on chemical composition, fermentation, metabolisable energy and organic matter digestibility of oak nut (*Quercus coccifera*) using Hohenheim *in vitro* gas production technique.

MATERIALS and METHODS Oak nut samples

The oak nut was collected in 2015 and ground before microwave irradiation. Three 100 gr of oak nut samples were subjected to microwave irradiation for 0, 2, 4, 6 and 8 minutes at a power of 900 W using domestic microwave. During the microwave irradiation oak nut samples were rotated to decrease in temperature gradients and to results in more uniform temperature distributions (Parnian et al. 2013).

Chemical analysis

Dry matter, crude ash, crude protein and ether extract contents of oak nut was determined according to procedures of AOAC (1990). Condensed tannin contents of oak nut were analyzed using the method described by Makkar et al. (1995). Condensed tannin was used as an external standard to obtain a standard curve. All chemical analyses were carried out in triplicate.

In vitro gas production

Gas productions of oak nuts were determined using the method described by Menke and Steingass. (1988). A 200 mg of oak nut samples were subjected to fermentation in 100 ml glass syringes containing 30 ml of buffered rumen fluid obtained from three fistulated ram fed with a diet containing of alfalfa (60%) and barley (40%). Gas production was determinate at 0, 3, 6, 12, 24, 48, 72 and 96 h after fermentation start. Total gas productions were corrected for blank incubation. Gas production data were fitted to the exponential equation $y = A (1-exp^{-ct})$ (Orskov and McDonald, 1979) where y is the gas production at time t; A is the total gas production, c is the gas production rate constant, t = incubation time (h).

Metabolisable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (IVOMD) of oak nut

samples were estimated using equation suggested by Menke and Steingass (1988) as follows:

ME (MJ/kg DM) = 0.72 + 0.1559GP + 0.068CP + 0.249EE

OMD (%) = 14.88 + 0.8893GP + 0.448CP +0.651CA Where

GP = 24 h net gas production (ml/200 mg),

- CP = Crude protein,
- EE = Ether extract (%)
- CA = Ash content (%).

Data on chemical composition, gas production and estimated parameters were subjected to one way of ANOVA. Significance between individual means was identified using the Tukey's Multiple Range test.

RESULTS and DISCUSSION

The effect of microwave irradiation on the chemical composition is given in Table 1. Microwave irradiation has no significant effect on the chemical composition except for dry matter and condensed tannin contents of oak nut. Microwave irradiation increased the dry matter contents whereas microwave irradiation decreased condensed tannins of oak nut. This result is consistent with findings of Kala and Mohan (2011) who found that microwave irradiation decreased condensed tannins of Mucuna pruriens var utilis bean. High level of moisture in feedstuffs will results in microbial growth which led to spoilage during the storage (Maheri-Sis et al. 2011b). It is well known that the moisture content of feedstuffs should be lower than %13-14 for safe storage. As can be seen from Table 1, dry matter contents of oak nuts are subjected to microwave irradiation for 4, 6 and 8 minutes are suitable for safe storage.

The effect of microwave irradiation on the gas production over time, their kinetics (c and A), ME and OMD were given in Table 2.

Although microwave irradiation has a significant effect on the gas production at early incubation times, it has no significant effect on gas production at late incubation times. At early incubation times gas production linearly increased with time of microwave irradiation. The increase in gas production at early incubation times is associated with increased amount of substrate for micro-organisms. It is well known that the amount of gas depends on the available substrate for micro-organisms. The more substrate there is, the more gas production occurs. Microwaves irradiation may have resulted in gelatinization of oak nut. As a result of gelatinization, the more starch would be available for microbial fermentation which led to more gas production at early incubation times.

	Microwav	e treatments (
Parameters	0	2	4	6	8	SEM	p
DM	61.64 ^e	71.35 ^b	86.07 ^c	94.45 ^b	95.85 ^a	0.338	0.000
CA	1.88	1.83	1.82	1.80	1.83	0.076	0.857
СР	2.96	3.01	3.00	3.01	2.91	0.199	0.820
EE	5.55	5.63	5.23	5.57	5.60	0.277	0.625
СТ	6.12 ^a	4.41 ^b	4.56 ^b	4.55 ^b	4.56 ^b	0.183	0.000

Table 1. The effects of microwave irradiation on the chemical compositions

^{a b} Means within a row with common superscripts do not differ (P>0.05); SEM : Standard error mean, NS. Not significant DM: Dry matter (%), CA: Crude ash (%), CP: Crude protein (%), EE: Ether extract (%), CT: Condensed tannin (%), *** P<0.001.

Table 2. The effects of microwave irradiation on in vitro gas production of oak nut (Quercus coccifera)

	Microwa	ve treatments					
Incubation time (h)	0	2	4	6	8	SEM	p
3	6.37 ^c	7.19 ^{bc}	9.66 ^{ab}	8.84^{ab}	10.48^{a}	0.937	0.001
6	11.11 ^b	13.16 ^b	17.28^{a}	18.10^{a}	19.33 ^a	0.973	0.000
12	20.98 ^c	26.33 ^b	30.44 ^a	30.85 ^a	32.09 ^a	0.688	0.000
24	53.26	49.97	49.97	52.44	52.85	1.326	0.077
48	61.28	60.05	62.52	62.52	63.34	1.666	0.371
72	63.55	63.55	65.20	65.61	66.01	1.875	0.569
96	63.97	64.38	67.26	66.43	67.67	1.783	0.210
Parameters							
с	0.045 ^b	0.048^{ab}	0.052^{ab}	0.054^{ab}	0.055 ^a	0.003	0.027
А	67.07	66.53	67.33	67.64	67.88	2.022	0.966
ME	10.03	9.53	9.53	9.90	9.96	0.210	0.098
OMD	64.80	61.83	61.80	63.93	64.36	1.199	0.082

^{ab} Means within a row with common superscripts do not differ (P>0.05); SEM : Standard error mean, NS. Not significant, A: total gas production (ml/200 mg DM), ME: Metabolisable energy (MJ /Kg DM); OMD: Organic matter digestibility (%), *** P<0.001.

This result is in agreement with findings of Khajehdizaj et al. (2013) who suggested that microwaves irradiation results in gelatinization of starch, which led to chemical and physical changes in the starch granules and facilitated starch availability for micro-organisms to ferment them.

In addition, the increased gas production at early incubation times would be associated with the decrease in condensed tannin contents of oak nut with microwave irradiation. As can be seen from Table 1, the microwaves irradiation decreased the condensed tannin of oak nut. It was suggested that the condensed tannin in feedstuffs may have detrimental effect when condensed tannin is higher than %5 of dry matter since condensed tannin combined with nutrients in feedstuffs, make it unavailable for micro-organism and animals (Kumar and Sing 1984). Condensed tannin can also affect the microbial and enzyme activities (Singleton 1981, Lohan et al. 1983, Barry and Duncan 1984, Makkar et al. 1989). Beside the gelatinization, in activations of some condensed tannin of oak nut with microwave irradiation make available more substrate for micro-organisms. The increase substrate availability may have resulted in the increased in gas production at early incubation times. The absence of significant increase in the gas production at the late incubation resulted in speculation that microwave irradiation has no significant effect on the cell wall contents of oak acorns.

Microwave irradiation has no effect on the estimated parameters such as A, ME and OMD whereas it increased (P<0.001) gas production rate (c) of oak nuts. As can be seen from Table 2 the increase in the gas production rate is possibly associated with increase in the gas production at early incubation times due possibly to increased gelatinization of starch and decreased condensed tannin. The effect of microwave irradiation on feedstuffs is not consistent. The effect of microwave irradiation varied with type of feedstuffs (Maheri-Sis et al. 2012, Parnian et al. 2013).

In terms of gas production, organic matter digestibility, metabolisable energy, the effect of microwave irradiation varied with type of feedstuffs (Parnian et al. 2013). Although gas production, organic matter digestibility, metabolisable energy, net energy for lactation and short chain fatty acids production of sorghum increased with microwave irradiation for 3, 5 and 7 min, microwave irradiation has no effects on the same parameters of wheat grain (Parnian et al. 2013).

CONCLUSION

Microwave irradiation has no effect on the chemical composition, metabolisable energy and organic matter digestibility except for dry matter, condensed tannin contents and fermentation rate. Therefore microwave irradiation can be used to dry oak nut with high moisture when harvested since microwave irradiation is a very fast method for drying of oak nut with high moisture. However the cost of drying of acorn with microwave irradiation should be tested before large implication.

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