

CHANGES OCCURRED IN FATTY ACIDS COPOSITION AND ANTIOXIDANT ACTIVITY OF GREEN SEASONIG SAUCE DURING MARINATING , COOKING AND STORAGE.

EI - Refai, A. A.; A. M.Hassan.; M. M. Rabie and E. M. Essa
Food Industries Dept., Faculty of Agric., Al-Mansoura Univ., Mansoura, Egypt

ABSTRACT

The aim of this study was to determine the effect of marinating, cooking and storage on antioxidant activity of a very popular marinating sauce , namely green seasoning sauce (GSS). GSS was prepared and thermal treated at 204 °C for 40 min.. placing of untreated sauce on the surface of beef was occurred. Gross chemical composition, chemical indices of oil extracted from sauce, fatty acid composition and antioxidant activity (DPPH) were determined in sauce only and in sauce after marinating of beef 5 hours.

Acid value , free fatty acids , peroxide value and thiobarbituric acid of the sauce were increased after marinating and cooking at 204 °C for 40 minutes. Concerning to fatty acid composition , it was observed that palmitic and stearic acids were the predominant saturated fatty acids , while oliec and linoleic constituted about 32 and 47 % respectively. The saturated fatty acids were increased after marinating . Antioxidant activity of sauce was increased from 88.5 to 90.4 % after cooking , but the final thermal process after 5 hrs marinating led to a reduction of antioxidant activity . Also , the storage of sauce at 5±1 °C for 2-4 months reduced antioxidant activity.

Keywords: Antioxidants, Fatty acids, Marinating sauce, Cooking process.

INTRODUCTION

Dietary antioxidants are either synthetic or natural compounds. Synthetic antioxidants such as butylate dhydroxy toluene (BHT) have been in use since the beginning of the past century. However, there is widespread agreement that, because of their potential health risks and toxicity, some synthetic antioxidants need to be replaced with natural antioxidants (Safer and Al-Nuhamish, 1999). The desire for new sources of safe and inexpensive antioxidants of natural origin has resulted in considerable interest in herbs and spices as sources of natural antioxidants (Yanishlieva *et al.*, 2006).

Even though spices and herbs are excellent sources of antioxidants, it is difficult to estimate typical amounts consumed, because spices are generally not consumed in large quantities, at least compared to fruits and vegetables. Instead, spices are used as ingredients, typically in relatively small amounts in recipes and formulations such as spice mixes and marinating sauces to enhance food flavor. Some marinating sauces for example, contain different combinations of herbs and spices forming very potent blends with very distinct flavors and are very popular in every day cuisine and diets. It is expected that these marinating sauces should be excellent sources of antioxidants, since their main ingredients are derived from herbs and spices. However, these sauces undergo different processing

methods during their production and are often used to marinate foods for different periods of time, and are exposed to various methods of cooking. All of these factors may potentially alter the antioxidant status of sauces significantly, and consequently the amount of antioxidants available to the consumer. Currently, there is little information available about the antioxidant status of many of these common herb- and spice-based marinating sauces, and what effect, if any, marinating and cooking have on their antioxidant status (Thomas *et al.* 2010). Consequently, the objective of this study is to determine the effects marinating and cooking on fatty acids profile and the antioxidant activities of popular herb- and spice-based marinating green seasoning sauce.

MATERIALS AND METHODS

Materials

Raw materials:

Herbs, spices, olive and sunflower oils were obtained from local market of Mansoura City, Egypt.

Meat: six rectal cuts of sirloin (250 grams) were obtained from the local market of Mansoura City.

Chemicals:

All chemicals and reagents which were used in this study were purchased from EL-Gomhoria Company, in Mansoura City.

Sauce preparation :

The green seasoning sauce consists of 35 gm chopped green onion (*Allium cepa*), 40 gm chopped parsley (*Petroselinum crispum*), 35 gm chopped celery (*Apium graveolens var. dulce*), 35 gm chopped white onion, 40 gm chopped sweet basil (*Ocimum basilicum*), 15 gm rosemary (*Rosemarin usofficinallis*), 15 gm grated ginger (*Zingiber officinale*), 20 gm chopped garlic (*Allium sativum*), 15 gm sunflower oil. Fresh sauce sample was dried at 70 °C for 6 hours to reduce its moisture content up to approximately 10%.

Preparation of samples:

Samples in this study were designed to carry out as follows.

a- Fresh marinating sauce namely ("sauce only") was prepared before and after thermal treatment at 204°C for 40 min in the oven of a regular kitchen oven.

b- The marinating sauce were placed on the surface of beef and through simple cracks marinating agents were penetrated. These samples were analyzed directly after preparation (control sample), after 5 hours as marinating period and after marinating period and cooking.

Oil extraction:

The partially dried sample was extracted with petroleum ether (1:1, w/v) over night under refrigeration as a cold extraction method.

The mixtures were filtered and the residues were re-extracted three times, the filtrates were collected in clean separated dried beaker, the solvent

was evaporated by electrical rotary evaporation and the oil sample was kept and stored in refrigerator at 4 °C until analysis.

Analytical methods:

Moisture, protein, fat and ash contents were determined as described by **AOAC (2005)**.

PV, AV and FFA% values were determined according to the modified procedure described by AOAC (2005). The PV was expressed as mill equivalent peroxide/kg oil or fat. The (FFA%) is applying the following equation. Free fatty acid % (as oleic acid) = acid value/1.99

TBA values were determined according to the method described by **Dobbs (1975)** using UV/VIS spectrometer, model:T80+ and absorbance was measured at 538nm. TBA value was expressed as mg. malonaldehyde/kg.

Fatty acids composition:

Sample of 50 mg of lipid was weighted in tube, and 50 ml of methanolic sulfuric acid (1 ml concentrated sulfuric acid and 100 ml methanol) and 2 ml of benzene were added. The tube was well-closed and placed in water bath at 90 °C for an hour and half. The tube was cooled, 8 ml water and 5 ml petroleum ether were added. Subsequently, the tube was strongly-shacked and the ethereal layer was separated out and evaporated to dryness. is showing the condition used for characterization of fatty acids by GC. Standard fatty acids (C₂-C₂₅) were previously injected with the same condition used by GC AOAC (2005).

Radical-scavenging activity effect (DPPH):

The free radical scavenging activities of the extracts were measured by the DPPH method proposed by Brand-Williams *et al.* (2000). Each extract was dissolved and diluted in 99% methanol. One milliliter of extract was added to 2 ml of DPPH (5.9 mg in 100 ml methanol) solution. The mixture was incubated for 30 min at room temperature and then the absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$ where A₀ was the absorbance of the control, and A₁ was the absorbance with the test compound.

RESULTS AND DISCUSSION

Gross chemical composition of green seasoning sauce before and after partially drying.

The prepared green seasoning sauce was partially dried at 70 °C for 6 hours and chemical composition was determined. The presented results in Table (1) show high moisture content (76.5%), and low protein, lipid and ash content (4.75, 5, 2 % respectively) , while after partially drying contents were moisture content was decreased from 76.5 to 9.5 %, and lipid, protein and ash relatively increased from 5 to 19, 4.75 to 20.2 and 2 to 9 %, respectively.

Table (1): Gross chemical composition of green seasoning sauce (GSS) as a percentage before and after partially drying at 70 °C for 6 hours .

Content	Sauce before partially drying	Sauce after partially drying
Moisture	76.5	9.5
Protein	4.75	20.2
Lipid	5	19
Ash	2	9

Chemical indices of extracted oil from green seasoning sauce (GSS):

Data presented in Table (2) prove that the green seasoning sauce before thermal process had a high level of acid number (7.23) and then highly free fatty acids % (3.63%). Both, peroxide and thiobarbituric acid values showed the same elevated initial values (9.04meq/kg oil and 0.647mg MAD/kg oil). This observation due to its high moisture content which enhances the reaction of oil hydrolysis.

Table (2): Acid values (AV), free fatty acids%(FFA), peroxide values(PV) and thiobarbituric acids(TBA) of extracted oil from green seasoning sauce (GSS):

Treatments	AV (mg KOH/g oil)	FFA% (as oleic)	PV (meq / kg oil)	TBA (mg MDA/kgoil)
Sauce only before cooking	7.23	3.63	9.04	0.647
Sauce only after cooking	8.46	4.25	10.5	0.959
Sauce and beef(zero time)	11.03	5.54	9.67	0.832
Sauce and beef (5h marinating)	12.63	6.34	12.2	0.909
Sauce and beef (5h marinating and cooking)	13.95	7.01	9.66	1.112

mg MAD : mg malonaldehyde

On the other hand, all values of AV, FFA% , PV and TBA values were increased in green seasoning sauce during cooking process at 204 °C for 40 min. .These results are in accordance with the results of Shahidi and Spurvey (1996), who noticed that autoxidation of oils and the decomposition of hydroperoxides increase in parallel with the temperature increases.

The percentage of increases which due to cooking process could be noticed at Fig (1A), where acid values and free fatty acids showed 17%; while peroxide and TBA values showed 16.15 and 48.22 % respectively, compared with control. These findings are in harmony with those obtained by Kambiz *et al.* (2003).

Also from Table (2) and Fig (1B) , it could be noticed that the FFA, acid, peroxide and TBA values were increased 52.55, 52.55, 7 and 28.6 % respectively, by adding green sauce on the beef compared with control. These increments attributed to high moisture contents of sauce which enhances the hydrolysis process. Also, the presence of meat which is highly vulnerable to oxidation reacting in rancidity and consequently off-flavor (Morrissey *et al.*, 1998).

Data tabulated in Table (2) and illustrated in Fig(1C) indicated that the marinating process (5 hours) increased all the aforementioned values comparing with either the sauce only before cooking or adding sauce to beef (zero time). This observation is in agreement with those reported by Rossell (1999).

The final cooking process (204 °C for 40 min), leads to rise the free fatty acid, acid and thiobarbituric acid (TBA) values because of the high moisture content and the high temperature (204°C) used, and the prolonging of cooking period (40 min) which helps to increase the hydrolysis process of oil, while the peroxide value decreased by 20%, compared with 5 hours marinating. This decrease resulted to the breakdown the peroxides .(Fig1D). This observations in agreement with those reported by Barbanti *et al.* (1994) and Kambiz *et al.* (2003), who stated that the presence of air and water during heat treatment effects the level of oil degradation.

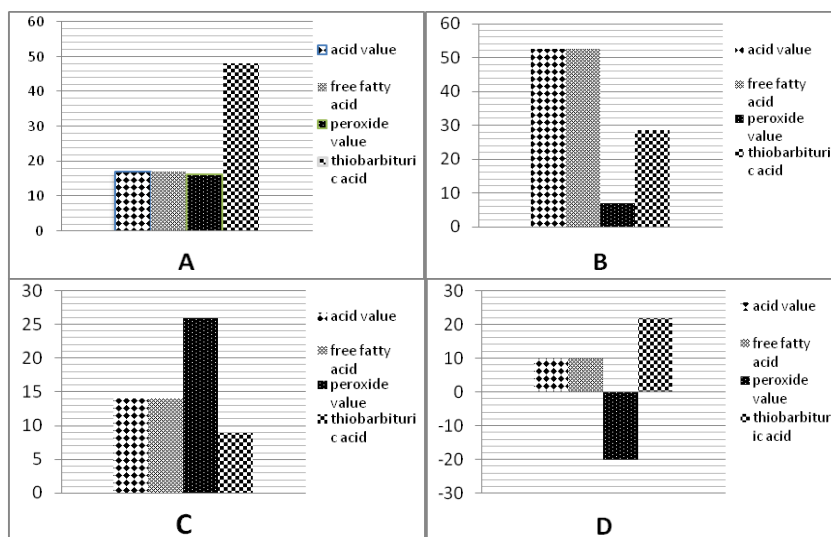


Fig (1): Effect of different treatments of green seasoning sauce (GSS)
A- sauce only after thermal process
B-adding sauce to beef (zero time)
C-marinating sauce (5h marinating)
D-marinating sauce (5 hours) and cooking

Fatty acids composition of extracted oil from green seasoning sauce (GSS).

The fatty acid profile of any oil, as is well known, is one of the main responsible factors for the high oxidative stability of the oil.

Data in Table (3) illustrated that the fatty acids identified in extracted oil of green seasoning sauce before thermal process. The total unsaturated and saturated fatty acids constitute about 80.71 and 18.62 %, respectively. The main saturated fatty acids were palmitic and stearic acids representing 9.04 and 5.68 %, respectively. But the main unsaturated fatty acids were oleic and linoleic acids (32.01 and 46.83 %, respectively). linoleic acid and oleic acid which are considered fatty acids which protect the body from cardiovascular diseases (CVD).

Table (3): Fatty acids profile (g per 100g)of extracted oil from green seasoning sauce (GSS).

Fatty acids %	Before cooking			After cooking	
	Sauce only	Sauce and beef (zero time)	Sauce and beef (5h marinating)	Sauce only	Sauce and beef (5h marinating)
Caprylic acid C8:0	0.15	---	0.03	1.22	0.02
Capric acid C10:0	0.05	0.06	0.08	0.33	0.08
Undecanoic acid C11:0	0.17	0.05	---	0.14	0.03
Lauric acid C12:0	0.29	0.30	---	2.36	---
Tridecanoic acid C13:0	0.61	1.05	0.58	0.36	0.48
Myristic acid C14:0	0.98	2.29	1.43	1.73	1.83
Pentadecanoic acid C15:0	0.62	1.06	0.56	0.84	0.87
Palmitic acid C16:0	9.04	18.39	19.14	13.40	21.49
Omargaric acid C17:	---	0.24	0.29	0.78	0.74
Stearic acid C18:0	5.68	10.14	12.78	8.61	13.50
Arachidonic acid C20:0	0.31	0.51	0.45	1.14	0.39
Heneicosanoic acid C21:0	0.72	0.51	0.22	3.22	0.10
Σ SFA	18.62	34.6	35.56	34.13	39.53
Tetradecanoic acid C14:1	0.25	0.45	0.17	0.40	0.19
14-pentadecanoic C15:1	0.24	0.49	0.27	0.42	0.49
Oleic acid C18:1	32.01	26.48	25.55	28.12	23.43
Linoleic acid C18:2	46.83	36.68	34.77	30.30	28.76
go Eicosenoic acid C20:1	0.24	0.23	0.24	0.88	0.28
Eurucic acid C22:1	0.87	---	---	1.30	0.06
Decosadinoic acid C22:2	0.27	---	---	1.17	---
USFAΣ	80.71	64.33	61	62.59	53.21
Total	99.69	98.93	96.56	96.72	92.74
B.V	4.25	1.85	1.71	1.83	1.49

Results in Table (3) and Fig (2A) indicate that the effect of thermal process on fatty acid profile of extracted oil of green seasoning sauce which resulted an increase in total saturated fatty acids (approximately 83.29 %) and mainly palmitic and stearic acids which increased by 48.23 and 51.58 % respectively, but the total unsaturated fatty acids were decreased by 22.45 % , and mainly linoleic and oleic acids which decreased by 35.29 and 12.15 % respectively, comparing to control sample (sauce only before cooking). These

changes leads to lowering biological value. These findings are in harmony with results obtained by Choe and Min (2007), who studied the hydrolysis, oxidation and polymerization of oils from common chemical reactions that occur during the frying process and cause the production of volatile and non volatile chemical compounds. These changes reduce the amount of polyunsaturated fatty acids and the amount of viscosity, color and free fatty acids increase.

It could be seen that adding sauce to beef (zero time) comparing it to control increased of some saturated fatty acids approximately by 85.82 % and mainly palmitic and stearic acids which increased by 100 and 78.52% respectively, while the total unsaturated fatty acids were decreased by 20.29 % , both linoleic and oleic acids were declined to 21.67 and 17.27 % respectively, this reduction was occurred as result of mixing sauce with beef process. These changes lowered the oxidative stability and biological value of the product. (Fig 2B).

On the other hand, the marinating process (5 hours) caused a reduction in the total unsaturated fatty acids (5.17 %), especially linoleic and oleic acids by 5.2 and 3.51 % respectively, while the total saturated fatty acids slightly increased (~2.77 %), particularly stearic and palmitic acids by 26 and 4 % respectively. William and List (1999).

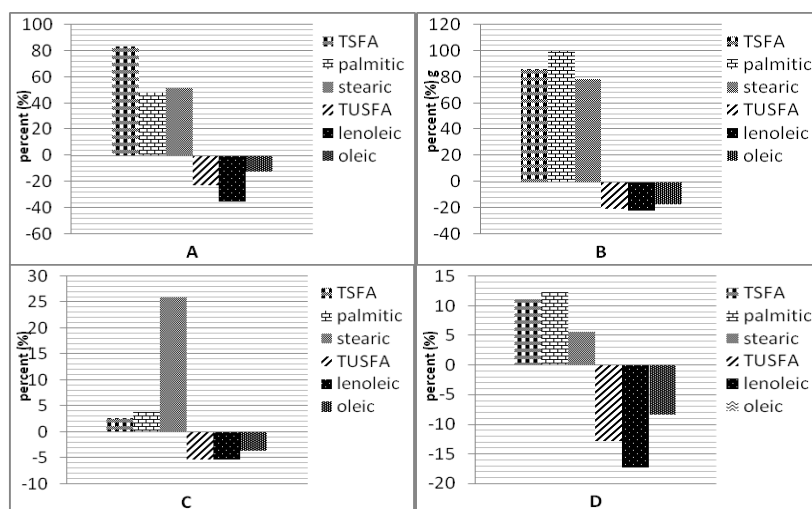


Fig (2): Percentage loss and gain of fatty acids profile of different treatments from green seasoning sauce (GSS).

A-sauce only after thermal process
C-marinating sauce (5 hours)

B- adding sauce to beef (zero time)
D-marinating sauce (5 hours) and cooking

From the same Table, data indicate that the effect of cooking process leads to increment the total saturated fatty acids by 11.16 % and mainly palmitic and stearic acids which increased by 12.27 and 5.63 % respectively, but its decreased the total unsaturated fatty acids by 12.77 % and the mainly unsaturated fatty acids which decreased were linoleic and

oleic acids by 17.28 and 8.29 % respectively, comparing with 5 hours marinating process. The used marinating sauce should be excellent sources of antioxidants , since their main ingredients are derived from herbs and spices. This sauce was exposed to cooking . The marinating and cooking may potentially alter the antioxidant status of studied sauce and consequently the amount of antioxidants available . Therefore, the status of fatty acid profile was not altered greatly as expected due the presence of antioxidants present in herbs consisting the sauce. These findings are in accordance with results obtained by Lim *et al.* (1993) .

Effect of cooking and storage on radical scavenging activity of green seasoning sauce :

The antioxidant activity in the green seasoning sauce under investigation is due to the phenolic compounds present in herbs and spices used to formulate this sauce. This sauce is exposed to cooking and is used to marinate foods for different periods of time . All of these factors may potentially alter the status of sauce and consequently the amount of antioxidants available to the consumer.

The scavenging efficiency of sauce under investigation was measured before and after cooking to determine its content of the stable free radicals and changes occurred during cooking and the results are presented in Table (4) and Fig.(3).

The results show that the highest scavenging efficiency was found in sauce after cooking (90.4 %). This means that cooking process of sauce may result in the formation of new phenolic compounds, which may enhance the antioxidant capacity of the sauce (Thomas *et al.*, 2010). After marinating , it was noticed that scavenging efficiency was reduced to 66.9 % . This explains that during marinating a proportion of antioxidant compounds were lost gradually and may be transferred to the food marinated. Also during storage of sauce at 5±1 °C for 2 and 4 months , the scavenging efficiency was reduced to 77.7 and 68.3 % , respectively (Table 4 and Fig 3).

These results indicate that marinating and storage reduced the antioxidant activity of green seasoning sauce , but cooking of this sauce increased the antioxidant activity . These results are in accordance with those of Ames *et al.*, (1995) , Stewart *et al.*, (1999) and Dewanto *et al.*, (2002).

Table (4): Radical scavenging activity of green seasoning sauce before and after cooking and storage at 5±1 °C.

Treatments	Antioxidant activity %
Sauce only before cooking	88.5
Sauce only after cooking	90.4
Sauce and beef (zero time)	70.9
Sauce and beef(5h marinating)	72.7
Sauce and beef(5h marinating +cooking)	66.9
After 2 months storage	77.7
After 4 months storage	68.3

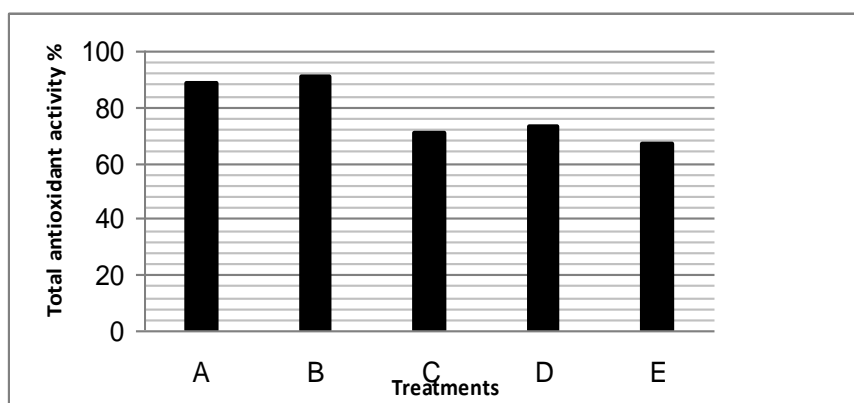


Fig (3): Changes in total antioxidant activities % DPPH of different treatments from green seasoning sauce .

Treatments:

A-sauce only before cooking

C-sauce and beef (zero time)

**B-sauce only after cooking
marinating)**

D- sauce and beef (5 hours

E-sauce and beef (5hours marinating and cooking)

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التغيرات التي تحدث للأحماض الدهنية و مضادات الاكسدة لصلصة التتبيل الخضراء اثناء التتبيل والطبخ والتخزين .

احمد عبدالعزيز الرفاعي، ابو بكر محمد حسن، ممدوح محمد ربيع واسماعيل محمد عيسى
قسم الصناعات الغذائية - كلية الزراعة - جامعة المنصورة - مصر

تهدف هذه الدراسة الى بيان تأثير عمليات التتبيل والطبخ والتخزين على نشاط مضادات الاكسدة في صلصة التتبيل الشعبية مايسمى بصلصة التتبيل الخضراء. وقد تم اعداد هذه الصلصة ومعالجتها حراريا على ٢٠٤م/٥ ٤٠ دقيقة. تم وضع الصلصة على سطح اللحم. تم تقدير التركيب الكيمياوي الاجمالي و المؤشرات الكيمياوية من زيت المستخلص من الصلصة ، تركيب الاحماض الدهنية ونشاط مضادات الاكسدة في صلصة المنفردة وكذلك عند تتبيلها مع اللحم لمدة خمس ساعات .

واوضحت النتائج زيادات في كل من رقم الحامض والاحماض الدهنية المنفردة ورقم البيروكسيد ومعدل الاكسدة الذاتية للدهن من الصلصة بعد التتبيل والطبخ على ٢٠٤م/٥ ٤٠ دقيقة. بالنسبة لتركيب الاحماض الدهنية لوحظ ان حامضي البالمتيك والاسثيريك كانت من الاحماض الدهنية المشبعة السائدة . بينما حامضي الاوليك و لينوليك شكلا حوالي ٣٢ ، ٤٧ % على الترتيب ، من مجموع الاحماض الدهنية غير المشبعة. وقد ادت عملية التتبيل الى زيادة الاحماض الدهنية المشبعة. وايضا اظهرت النتائج ان عملية الطبخ ادت الى زيادة نشاط مضادات الاكسدة بنسبة ٨٨.٥-٩٠ % ، ولكن ادت عملية الطبخ النهائي بعد عملية التتبيل خمس ساعات الى انخفاض في معدل نشاط مضادات الاكسدة. وايضا اظهر تخزين الصلصة على ١±٥ لمدة ٢-٤ اشهر انخفاضا في نشاط مضادات الاكسدة.