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EFFECT OF DRYING PROCESSES ON THE ANTIOXIDANT PROPERTIES OF TOMATO SEEDS.

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ABSTRACT

In this research, one variety of tomatoe seeds (*Lycopersicon esculentum Mill*.), was used to study the effects of different drying processes, (freeze-drying (FD) and air-drying (AD), on the antioxidant properties of tomato seeds. The quantitative analysis of antioxidative components showed that fresh tomato seeds had highest amount of ascorbic acid but lowest amount of total flavonoids. While air dried tomato seeds had the highest content of vitamin E, total phenolics and total flavonoids. On the other hand the air dried and freeze-dried tomato seeds had an equal content of lycopene nearly. The analysis of the methanolic extract from freeze-dried tomato seeds gave the highest reduction activity and $H_٢O_٢$ scavenging activity while the fresh tomato seeds had the lowest.

Keywords: Antioxidant properties; Freeze-drying; Air-drying; Lycopene; Tomato seeds.

INTRODUCTION

Tomato fruit is a versatile vegetable that is consumed fresh as well as in the form of processed products. More recently, there has been renewed attention given to the antioxidant content of tomatoes. Many epidemiological studies suggested that regular consumption of fruits and vegetables, including tomatoes, can play an important role in preventing cancer and cardiovascular problems (Giovannucci ١٩٩٩, Heber ٢٠٠٠ and Rao and Agarwal, ٢٠٠٠). Tomato components like lycopene, phenolics, flavonoids, ascorbic acid and vitamin E are mainly responsible for the antioxidant capacity of raw tomatoes and processed tomato products (Beutner *et al*., ٢٠٠١, Leonardi *et al*., ٢٠٠٠ and Stewart *et al*., ٢٠٠٠). Although the most worldwide produced tomatoes are used in the production of tomato paste, as the main ingredient in different processed tomato products such as ketchup, sauces, and soups (Sanchez *et al*., ٢٠٠٣), significant amounts of tomato fruits are consumed in the fresh state, as salads, or after cooking at home. However, some consumers remove the skin and seeds of tomatoes before eating them as raw fruit, because they are indigestible and contain low levels of nutrients. Furthermore, approximately one-third of the total weight of tomatoes in the form of skin and seeds is discarded during processing of tomatoes into paste (Al-Wandawi *et al*., ١٩٨٥).

In most of the previous studies, antioxidants have been measured, mainly in, whole tomatoes or processed tomato products (Lavelli *et al*., ٢٠٠٠, Martinez-Valverde *et al*., ٢٠٠٢ and Raffo *et al*., ٢٠٠٢). While Stewart *et al*., (٢٠٠٠) reported that the majority of the flavonols in tomatoes are present in the skin. Similarly, Sharma and Le Maguer (١٩٩٦) observed that most of the lycopene was associated with the skin and water insoluble fraction of the

tomato pulp. George *et al*. (٢٠٠٤) showed that there is a lack of information on the levels of antioxidants in the seed fraction of tomatoes, and this could be an important contributor to the antioxidant activity of tomatoes. In general, limited data are available on the contribution of the different fractions (skin, pulp and seeds) towards the total amount of the antioxidant components and antioxidant activity of tomatoes. Therefore, it is difficult to assess the health benefits of including the skin and seeds of tomatoes during home consumption or the production of processed products.

From the above-mentioned reports, it was observed that nutritional value of tomato seeds could be increased through different types of processing. In this study, two drying processes, freeze-drying and air drying treatments, were carried out to process tomato seeds. The drying effects on tomato seeds antioxidative properties represented by the amount of ascorbic acid, vitamin E, total phenolics, total flavonoids, and lycopene along with antioxidant activity represented by total reduction activity and H_2O_2 scavenging activity, were investigated and discussed.

MATERIALS AND METHODS

Materials

Tomato samples

Fresh tomatoes (Hybrid ٤٤٤) were taken from the Agric. Research center, Abnub city, Assuit Governorate during the Summer season ٢٠٠٩.

Methods

Sample Preparation.

The tomato seeds were collected during processing of tomato products. The seeds were treated as follow:-

١- Air drying, for ٢٤ h at ٥٠° C by using electrical drying oven. (Model D-٦٣٤٥٠, Hanau, Germany).

٢- Freeze-drying, for ٢٤ h by using freeze-drier (Model Inshin, HG-٣٠٨٥, Koria). After that the dried seeds were milled and packaged in polyethylene pags. All samples were homogenized and stored at -١٨° C until analysis (Chang *et al*., ٢٠٠٦).

Quantitative analysis of antioxidative components

Ascorbic acid

Ascorbic acid was determined according to the method described by Sahlin *et al.*, (٢٠٠٤). The results were expressed as mg/١٠٠ g fresh weight (FW).

Total phenolics

Total phenolic compounds in tomato seed samples were determined spectrophotometrically using Folin–Denis reagent (AOAC, ١٩٩٥). The methanolic extracts (٠,١ ml) of tomato seed samples were diluted with distilled water (٧٥ ml) in a volumetric flask. Folin-Denis reagent (٥ ml) was added, and the contents of the flask were mixed thoroughly. After ٣ min, ١٠ ml of Na_2CO_3 solution (١٠ g / ١٠٠ ml) was added and finally quantified to ١٠٠ ml with distilled water. The mixture was allowed to stand for ٣٠ min with intermittent shaking. The blue color was measured by spectrophotometer at

٧٥٠ nm. The concentration of total phenolic compounds in tomato seed samples was determined compared with the absorbance of standard tannic acid at different concentration.

Determination of phenolic acids:

The HPLC analysis of phenolic acids were carried out on a HPLC apparatus consisting of Merck-Hitachi L-٧٤٥٥ diode array detector (DAD) and pump L-٧١٠٠ equipped with D-٧٠٠٠ HSM Multisolvent Delivery System. The separation was performed on a Li ChroCART® ١٢٥-٣ Purospher® RP-١٨ (٥ µm) Merck column. Column oven temperature was set to ٣٠°C. ٨٠٪ acetonitrile in ٤,٥٪ formic acid (reagent A) and ٢,٥٪ acetic acid (reagent B) were used as an eluent. The flow rate was ١ ml/min. The concentration of reagent A was stepwise increased to reach ١٥٪ after ٧min, ٢٠٪ after ١٥ min and ١٠٠٪ after ١٦ min. After ١٠ min of elution the concentration of reagent A was reduced to ٠٪ to stabilize the column. During analysis the solvent was degassed in Merck degasser. Data logging were monitored at wavelength ٢٨٠ nm. Retention times and spectra were compared to those of pure standards (Goupy *et al*., ١٩٩٩).

Total flavonoids

The aluminium chloride colorimetric assay was used for total flavonoids determination, as described by Marinova (٢٠٠٥**)**. Extraction of flavonoids in the samples (n=٣) was achieved by homogenizing ٢ g of the sample in ٥٠ mL distilled water in pestle and mortar. The mixture was transferred into a rotary shaker for ١٢ h to ensure full extraction. Thereafter, the mixture was filtered and the filtrate (extract) made up to ٥٠ mL. Precisely, ١ ml of extracts or standard solution of catechin (٢٠, ٤٠, ٦٠, ٨٠ and ١٠٠ mg/ L) was added to test tubes containing ٤ ml of redistilled water. To this mixture ٠,٣ ml of ٥٪ $NaNO_٢$ was added. After ٥ min, ٠,٣ ml ١٠٪ $AlCl_٣$ was added. Immediately, ٢ ml ١M NaOH was added and the total volume was made up to ١٠ ml with redistilled water. The solution was mixed thoroughly and the absorbance of both the samples, blank and standard, were estimated at ٥١٠ nm using UV–Visible spectrophotometer Model UV ١٦٠١ version ٢,٤٠ (Shimadzu). Total flavonoids content was expressed as mg catechin equivalents.

Lycopene

Lycopene content of tomato seed extracts was determined using a colorimetric method by Rao *et al*., (١٩٩٨). Lycopene from tomato products was extracted with hexane, methanol, and acetone together with a volume ratio of ٢:١:١ respectively for ١ h. Absorbance of the extract at ٥٠٢ nm was measured using spectrophotometer against the blank extract solvent.

Vitamin E

Two grams of sample was weighed into centrifuge tubes. Successively, ١ ml of distilled water, ١ ml of ethanol, ١ ml of methyl *tert*-butyl ether (MtBE), and ١ ml of petroleum ether were added. After each addition, the tubes were shaken for ٣٠ sec. Then, the samples were centrifuged (٥٠٠٠ rpm, ٥ min), and the upper layer was transferred into a flask. The extraction with ١ ml of MtBE and ١ ml of petroleum ether was repeated twice. The combined extracts were dried under vacuum at ٣٠° C in a rotary evaporator. The

residue was dissolved in ٢ ml of mobile phase (*n*-hexane: MtBE; ٩٦: ٤, v/v) and then centrifuged (١٤٠٠٠ rpm, ٥ min). The resulting solution was analyzed for vitamin E by HPLC at ٥٠° C. Tocopherols are separated on a column packed with ٥ µm LiChrosorb RP١٨ (Merck) (١٢٠ x ٤.٦ mm), with methanol/water (٩٨:٢) as the mobile phase, flow rate: ١,٥ mL/min. (Balz *et al.,* ١٩٩٢).

Antioxidant activities assays

Total reduction activity by $Fe^{٣+}$- $Fe^{٢+}$ transformation

The reducing activity of tomato seed samples was determined by the method of Oyaizu (١٩٨٦). The capacity of tomato seed samples to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the absorbance at ٧٠٠ nm after incubation. Increased absorbance of the reaction mixture indicates greater reduction capability.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of tomato seed samples was determined according to the method of Ruch *et al*., (١٩٨٩**).** A solution of $H_٢O_٢$ (٤٠ mM) was prepared in phosphate buffer (pH ٧,٤). Sample extract, at the ٣٠ µg ml-١ concentration in ٣,٤ ml of phosphate buffer, was added to an $H_٢O_٢$ solution (٠,٦ ml, ٤٠ mM). The absorbance value of the reaction mixture was recorded at ٢٣٠ nm against blank solution which contained the phosphate buffer without $H_٢O_٢$.

RESULTS AND DISCUSSION

Effects of drying treatments on antioxidants content of tomato seeds.

Lycopene, total phenolic and total flavonoids compounds of tomato seeds samples were determined and the results are shown in Table (١) and Fig. (١). From these results, it was found that the antioxidants compounds detected in air dried and freeze dried tomato seeds were similar with values: ٠,١٨٣ and ٠,١٨٤ mg lycopene; ٥,٧٦ and ٥,٧١ mg total phenolics; and ٧,٥٨ and ٧,٤٩ total flavonoids / ١٠٠ gm wet weight, respectively. These values were higher than that found in fresh tomato seeds (٠,٠٧٦ mg lycopene; ٢,٢٨ mg total phenolics; and ٣,٠٤ total flavonoids / ١٠٠ gm wet weight).

Table ١: Lycopene, Total phenolics and Total flavonoids contents of tomato seeds samples.

Sample	Lycopene*	Total phenolics**	Total flavonoids***
Fresh tomato seeds	٠,٠٧٦	٢,٢٨	٣,٠٤
Air dried tomato seeds	٠,١٨٣	٥,٧٦	٧,٥٨
Freeze-dried tomato seeds	٠,١٨٤	٥,٧١	٧,٤٩

***Calculated as mg lycopene /١٠٠ gm wet weight**
****Calculated as mg tannic acid / ١٠٠ gm wet weight.**
*****Calculated as mg catechin / ١٠٠ gm wet weight.**

Dewanto *et al*. (٢٠٠٢) reported that the total phenolics did not change significantly during thermal processing of tomatoes. In contrast, our results showed an increase in total phenolics and total flavonoids by freeze drying and air drying processes. It is possibly due to the liberation of phenolic and flavonoids compounds from the matrix during drying processes.

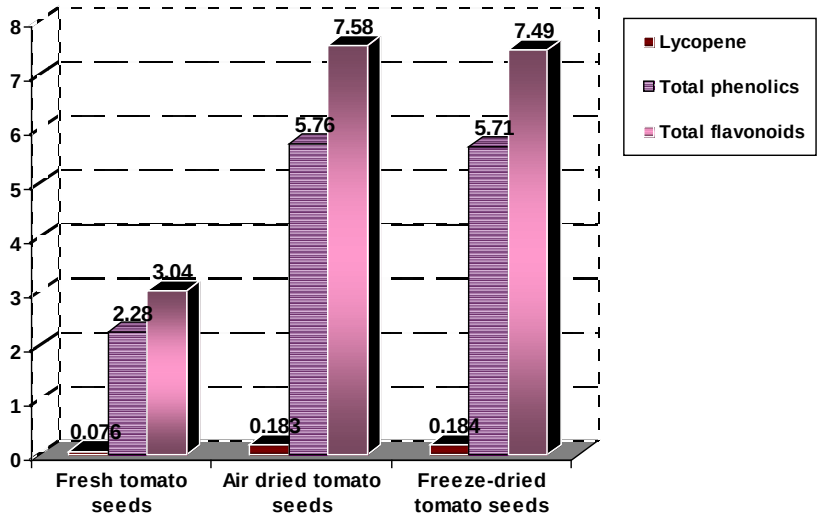

Fig. ١: Diagram of lycopene, Total phenolics and Total flavonoids contents of tomato seed samples

Polyphenolic compounds in tomato seeds.

HPLC analysis of methanol extract used in our study identified four polyphenols as major constituents including protochatechin, chlorogenic, ferulic and catechin, consistent with literature reports (Chang *et al*., ٢٠٠٦).
The composition of polyphenolic acids in tomato seeds samples are quantified in Table (٢). Protocatechin, catechin, caffeine, gallic and chlorogenic acids are the major constituents of polyphenolic compounds in tomato seed samples.

Table ٢: Phenolic acid contents of tomato seed samples*.

Sample	cinnamic	caffein	chlorogenic	chrisin	ρ-coumaric	Ferulic	vanillic	caffeic	catechin	gallic	naringenin	catechol	Protocatechin
Fresh tomato seeds	١,٢٣	٥,٨٤	٢,٦٧	٠,١١	٠,١١	٢,١٩	١,٤٧	٠,٧٩	٧,٩٨	٤,٤٢	٠,٣٧	١,٤٥	٩٦,٦٣
Air dried tomato seeds	١,٠٣	١٩,٧٣	١,٤١	٠,١١	٠,٣٨	٨,٦٧	٧,٩٩	٠,٩٦	٤,٠٩	١١,٠٩	١,٧٦	٨,١٥	٢١٦,١٦
Freeze-dried tomato seeds	١,٠٦	١٩,٥٠	١,٠٢	٠,٥٣	٠,٦١	١٤,٣٩	٧,١٣	٠,٥٩	١,٧٩	٢٠.٣٦	٠,٩٤	١٢,٦٧	٢٤٢,٧٠

***Calculated as mg acid / ١٠٠ gm wet weight.**

From the data in Table (٢), it is clear that the quantity of protocatechin, catechol, gallic, caffeine, ferulic and ρ-coumaric acid increased in dried samples as a result of drying process. Freeze dried tomato seeds had the highest amounts of protocatechin: ٢٤٢,٧٠; gallic: ٢٠,٣٦; ferulic: ١٤,٣٩ and catechol: ١٢,٦٧, as compared with air dried and fresh tomato seeds

(protocatechin ٢١٦,١٦, ٩٦,٦٣; gallic ١١,٠٩, ٤,٤٢; ferulic ٨,٦٧, ٢,١٩ and catechol ٨,١٥, ١,٤٥ mg acid / ١٠٠gm wet weight, respectively.

The air dried and freeze dried tomato seeds contained a higher contents of vanillic, naringenin and *ρ*-coumaric than fresh tomato seeds. On the other hand, the contents of catechin, cinnamic and chlorogenic acids were decreased in air dried and freeze dried tomato seeds as compared with fresh tomato seeds.

The increase in polyphenolic acids in air dried and freeze dried tomato seeds is due to the release of polyphenolics from the tomato seeds during drying. It is clear that the drying process might accelerate more bound phenolic compounds releasing from the breakdown of cellular constituents (Chang *et al*., ٢٠٠٦).

The results of this study point to that the changes in levels of all polyphenol classes and consequently the changes in the antioxidant activity of these compounds may be due to the effect and function of drying processes on tomato seeds.

Ascorbic acid and Vitamin E contents of tomato seeds samples.

Table (٣) and Fig. (٢) show the results obtained from the analysis of ascorbic acid and α-tocopherol in fresh and dried tomato seeds. The lowest values of ascorbic acid (٤,١٨ and ٤,٥٤ mg) were found in the air dried and freeze dried tomato seeds, while the fresh tomato seeds showed higher value of ascorbic acid (٩,١٥ mg /١٠٠ gm wet weight). Gregory (١٩٩٦) proposed that the loss of ascorbic acid was primarily due to chemical degradation involving oxidation of ascorbic acid to dehydroascorbic acid, followed by hydrolysis to ٢،٣- diketogulonic acid and further polymerization to form other nutritionally inactive products. Heating process is supposed to speed up ascorbic acid oxidation. Accordingly, air drying process resulted in high loss of ascorbic acid in the investigated samples.

Table ٣: Ascorbic acid and Vitamin E contents of tomato seeds samples.

Sample	Ascorbic acid*	Vitamin E**
Fresh tomato seeds	٩,١٥	٢,٠٨
Air dried tomato seeds	٤,١٨	٦,٩٥
Freeze-dried tomato seeds	٤,٥٤	٤,٣٣

*** Calculated as mg ascorbic acid / ١٠٠ gm wet weight.**
****Calculated as mg α-tocopherol / ١٠٠ gm wet weight.**

Regarding to vitamin E content among three tomato seeds examined, air dried contained the highest level (٦,٩٥ mg) followed by freeze dried tomato seeds (٤,٣٣ mg) and fresh seeds (٢,٠٨ mg α-tocopherol / ١٠٠ gm wet weight). Seybold *et al*., (٢٠٠٤) showed that, the heating of tomato products led to a significant rise (٥١-٧٣٪) in α-tocopherol content on both wet and dry weight bases. The increase in α-tocopherol contents due to tomato processing is a result to evaporation of water during thermal processing and α-tocopherol was released from its binding sites as a consequence of thermal treatment.

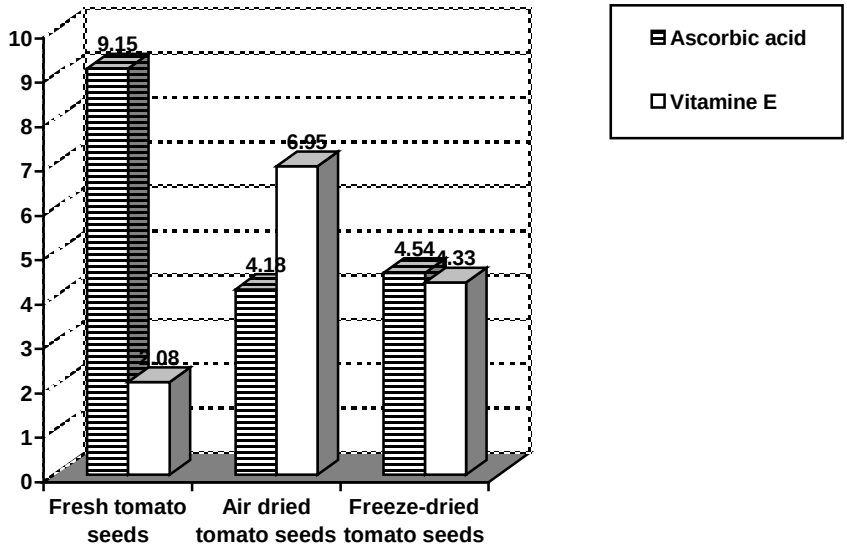

Fig. ٢: Ascorbic acid and Vitamin E contents of tomato seeds samples

Antioxidant activity of tomato seeds samples

Total antioxidant activity is a measure of the capacity of substances extracted from the food matrix to delay the oxidation process in a controlled system (Cao *et al*., ١٩٩٦; Miller and Rice-Evans ١٩٩٧; Fogliano *et al*., ١٩٩٩; Pellegrini *et al*., ٢٠٠٠). Antioxidative activity observed in tomato seeds samples is shown in Table (٤) and Fig. (٣).

Table ٤: Antioxidant activities content of tomato seeds samples.

Sample	Total reduction activity*	H_2O_2 scavenging activity**
Fresh tomato seeds	٢,٢٧	٨,٨٤
Air dried tomato seeds	٥,٢٤	١٨,٠٧
Freeze-dried tomato seeds	٧,٦٢	٢٣,٧٣

***Calculated as reducing ferric ions / ١٠٠ gm wet weight.**
****Calculated as scavenging H_2O_2 molecules / ١٠٠ gm wet weight.**

The contents of antioxidant activity as measured by the total reduction activity method were ٢,٢٧, ٥,٢٤ and ٧,٦٢٪, for fresh, air dried and freeze dried tomato seeds, respectively. Meanwhile the contents of antioxidant activity measured by Hydrogen peroxide scavenging activity method were in the same order but with higher values; ٨,٨٤, ١٨,٠٧ and ٢٣,٧٣٪ for fresh, air dried and freeze dried tomato seeds, respectively. The higher activities of freeze dried tomato seeds could be due to their highly antioxidant contents (lycopene and phenolic) compounds and highly antioxidative activity of polyphenols and flavonoids.

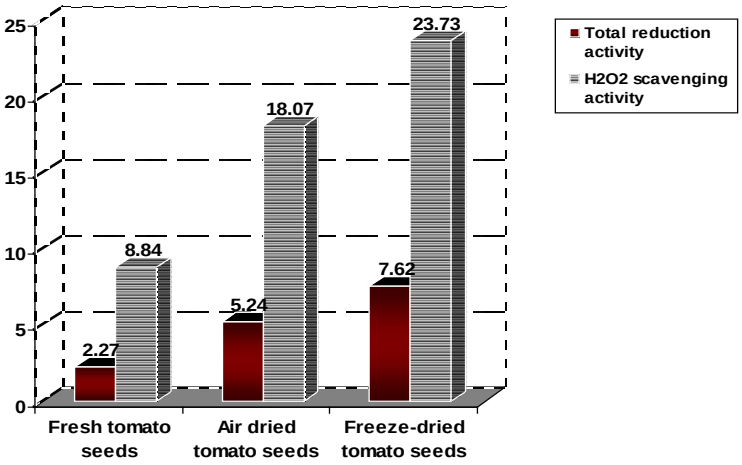

Fig. ٣: Antioxidant activities content of tomato seeds samples.

Conclusion

This study suggests that the seed fraction of tomato is a very rich source of antioxidant compounds and the incorporation of the seeds fraction during home consumption or processing could lead to increasing in the amount of all the major antioxidants in the final product. Therefore, removal of seeds during home cooking or processing resulted in a loss of their potential health benefits. Consumer demand for healthy food products provides an opportunity to develop foods rich in antioxidants as new functional foods. By adopting slight changes during processing, the antioxidant and nutrient composition of the final products can be increased, and a valuable reserve of antioxidants would be optimally utilized. So at the end we can conclude that the seeds fraction of tomatoes could be used as a value added ingredient in other food products.

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تأثير عمليات التجفيف على خصائص مضادات الأكسدة فى بذور الطماطم.

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فى هذا البحث تم استخدام نوع واحد من بذور الطماطم لدراسة تأثير عمليات التجفيف المختلفة (التجفيد والتجفيف الهوائى) على خصائص مضادات الاكسدة لبذور الطماطم. أظهر التحليل الكمى لمكونات مضادات الأكسدة أن بذور الطماطم الطازجة احتوت على أكبر كمية من حامض الاسكوربيك وأقل كمية من المركبات الفلافونيدية. بذور الطماطم المجففة هوائيا احتوت على أكبر كمية من فيتامين ه والمركبات الفينولية الكلية والمركبات الفلافونيدية الكلية. بذور الطماطم المجفدة والمجففة هوائيا احتوت على نفس الكمية تقريبا من الليكوبين. فى تحليل القدرة على الاختزال وكذلك القدرة على التخلص من فوق اكسيد الهيدروجين أعطى المستخلص الميثانولى لبذور الطماطم المجفدة أعلى قيم بينما بذور الطماطم الطازجة أعطت اقل قيم.

الكلمات المفتاحية: خصائص مضادات الأكسدة – التجفيد – التجفيف الهوائى – الليكوبين - بذور الطماطم.

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