Effects of sun and freeze-drying techniques on molecular, fatty acid and therapeutic properties of fermented goat milk product

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Abstract This study investigated the effect of sun drying (Sd) and freeze drying (Fd) on the chemical, nutritional and biological properties of either unsalted (Us) or salted (Sa) Jameed produced from goat milk. The products were characterized by measuring the chemical, physical and biological properties. SDS-PAGE was used to characterize the effect of processing conditions on protein subunits. Major new bands were found in SDS-PAGE of Jameed prepared by SdUs and FdUs from goat milk but not from that prepared by SdSa and FdSa. Preparation of Jameed by with or without salt treatments of Jameed by sun drying enhances the contents of short chain fatty acids. Result showed that the preparation of Jameed by SdUs decreased the content of caprylic acid. That prepared by sun drying and with or without salt increased the stability, shelf life and inhibitory activities of ACE and α -amylase. The optimum color values were found in Jameed prepared by FdSa. Different processing treatments influenced content of all fatty acids except for margaric and oleic acid.

Keywords Jameed · Fermented dairy product · Freeze drying · Sun drying · Salt

Introduction

Jameed is a traditional dairy product made and consumed in a number of Middle Eastern countries, particularly Jordan, Egypt, Syria and Iraq (Abu-Lehia 1988). It is made using traditional methods by most Jordanian families and has a long history in Bedouin culture. Jameed forms a major component of Bedouin diets, mainly in the preparation of their main dish (i.e. mansaf - lamb cooked in a sauce made from Jameed, served with rice) (Sawaya et al. 1984). Jameed is produced mainly in the spring when the amount of milk available (produced by sheep, goat, cow milk) exceeds the quantity required for immediate consumption. Preparation of Jameed allows surplus milk to be stored until required (Al-Mohizea et al. 1988).

Jameed can be classified as a shelf-stable cheese product produced by fermenting and drying milk. It is shelf-stable because it has a combination of low moisture and pH, high salt content, and contains lactic acid bacteria that decrease the growth of pathogenic microorganisms (McCarthy and Wood 1985). Jameed can be kept for years at an ambient temperature without spoiling and losing its nutritional value. Jameed has been reported to have an excellent nutritional value due to high protein (57.02 %) and fat (22.25 %) contents. The composition of Jameed varies due to differences between milk in different animal species, animal feed, processing method and milk production stage (i.e. lactation cycle). From a quality point of view, Jameed should not contain less than 15 % moisture (JISM 1997) to minimize microbial spoilage and prevent any unwanted physical and chemical changes from occurring during storage (Koç et al. 2008; Krokida and Marinos-Kouris 2003).

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Traditionally, Jameed is made from a heated, fermented dairy product (yoghurt) milk and drained using cheese cloth bags followed by salting, pressing and drying in the sun to produce the final product (Al Omari et al. 2008). Jameed prepared using traditional methods can become contaminated by spoilage microorganisms, soil, hair and insect fragments from the air, animals or the hands of the manufacturer. Other processing factors can affect over all Jameed quality such as milk composition after churning, irregular and slow growth of lactic acid bacteria, growth of non-lactic acid bacteria, and poor control of fermentation temperature (Jumah et al. 2000). Control over the fermentation and drying conditions is essential in order to produce a uniform and acceptable Jameed product.

Drying involves a reduction of moisture content to reduce or eliminate microbial growth; however, unwanted reactions and changing in physiochemical properties may take place during the drying of Jameed. These changes will be discussed in this paper. During Jameed making, salt is added to ensure the long shelf life. Several factors related to manufacturing and environmental conditions play an important role in the safety and shelf life of Jameed including the low-water activity, high salt content, level of lactic acid, and low pH (less than 4.0). The presence of salt and lactic acid in Jameed prevents the growth of pathogenic microorganisms, though excess salt intake is an important health risk.

Drying process of Jameed has many changes in the physical, nutritional, quality and chemical composition. The method we used to prepare Jameed involved using traditional methods as these may enhance the chemical, physical and biological properties due to the growth of microorganisms including bacteria, mold and yeast. The sun drying technique was used to enhance the quality of Jameed including flavor, aroma and texture due to the growth of bacteria and mold. Freeze-drying may be used in food industries in the production of high quality, nutritional and biological properties of food products. The main objective of this study was to evaluate the effect of sun and freeze drying techniques on the chemical, nutritional and biological properties of Jameed prepared from goat milk. The specific objectives of this study were to: (i) Evaluate the effect of drying techniques and salt addition on the chemical and physical properties of Jameed from goat milk; (ii) Evaluate the effect of drying techniques and salt addition on the biological properties of Jameed from goat milk (antioxidant, ACE and α -amylase enzymes).

Materials and methods

Materials

Milk samples from different sources were blended together to obtain a uniform starting material Jameed was made (Al-Karak city, Jordan) during summer of 2011. Milk was pasteurized and

Jameed was made according to the traditional Jordanian method (Fig. 1). Sun dried Jameed was prepared by placing Jameed balls (diameter=100–120 mm) on trays and then drying in the shade for 24 h followed by sun drying for 15 days (temperature range from 35 to 40 °C during day time). Freeze dried Jameed was prepared by drying at −55 °C and 10 mTorr for 48 h (Model LFD-5508, Republic of South Korea). The dried Jameed was stored at −18 °C until required for analysis.

Sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE)

SDS-Page was conducted using a mini-protein electrophoresis unit (Bio-Rad, 041BR57072, USA) using the method described by Laemmli (1970) with some modifications. Freeze-dried Jameed (0.1 g) was dissolved in 1 mL sample buffer mixture of 2 % glycerol, 0.5 M β -mercaptoethanol, 10 % SDS, 0.5 M Tris HCl (pH 6.8) and 0.1 % bromophenol blue. The sample buffer and Jameed were heated at 95 °C for 15 min. 25 μ L of samples and standards were loaded onto the electrophoresis gel and electrophoresis was performed at a constant current of 15 mA per gel/ for 1.5–2 h. To allow visualization of the protein bands, the gels were stained with a solution of 70 % water, 0.1 % Coomassie Brilliant Blue R-250, 20 % methanol and

Fig. 1 The processing scheme for preparation of Jameed from goat milk

10 % acetic acid then destained using a solution of 70 % water, 20 % methanol and 10 % acetic acid.

Color measurement

Sample color was measured using a Minolta colorimeter CR-300 (Ramsey, N.J., U.S.A.) and the results recorded in the $L^* a^* b^*$ color system. The $L^* a^* b^*$ color system consists of a luminance or Lightness component (L^*) and 2 chromatic components: the a^* component for green ($-a^*$) to red ($+a^*$) and the b^* component for blue ($-b^*$) to yellow ($+b^*$) colors. Values of the white standard were $L^*=97.10$, $a^*=+0.13$, $b^*=+1.88$. Color was measured at two positions on both sides of the sample (ground to form a powder). Three Jameed samples were measured for each treatment, and the measurements were averaged.

Lipid extraction, methylation and fatty acid determination

Lipid extraction

Lipid was extracted from the Jameed using the method of Folch et al. (1957) with some modifications. Jameed samples (20 g) were mixed with distilled water (20 mL). The slurry was then mixed with a methanol and chloroform solution (200 mL; 1:1 of v/v) and homogenized in a blender (Model WPB80BC, USA) for four min. Chloroform (100 mL) was added to the mixture and homogenized in the blender for further 2 min followed by filtration through filter paper (101FAST Qualitative Filter paper, China). The filtrate was recovered, and 100 mL of 1 M KCl was added to the filtrate. The solution was shaken for 2 min.

The lipid extract was held at 4 °C for 24 h until there was a clear upper layer. The slurry was dried with anhydrous Na_2SO_4 and filtered to separate proteins. The chloroform phase (the upper liquid layer) was evaporated at 50 °C using a rotary evaporator (Germany). The lipid extract was stored at −18 °C until required for analysis.

Methylation

The lipid extract sample (50 mg) was dissolved in hexane (1 mL) by mixing for 1 min. Potassium hydroxide (100 μL of a 2 M solution) prepared in anhydrous methanol, and acetic acid (100 μL) were added and mixed for 30 s (Christopherson and Glass 1969). An aliquot of this solution (0.25 mL) was methylated by adding 1.5 mL of HCl/methanol (5 % w/v), and 2 mL of hexane at 80 °C for 60 min.

Fatty acid determination by gas chromatography (GC)

A gas chromatograph (GC-2010, Shimadzu, Japan) fitted with a flame ionization detector was used to analyze and quantify

the content of individual fatty acids using the method of Ereifej et al. (2011) with modifications. Separation was performed on a Supelcowaxs-10 fused silica capillary column (60 m x 0.25 mm internal diameter; 0.25 μm film thickness; TR-CN100, Supelco Inc., Bellefonte, PA). The temperature profile in the GC during analysis was: hold at 140 °C for 1 min, then heat to 240 °C at 4 °C/min. The injector and detector were both held at 250 °C. The flow rate of He was 1.67 mL/min. A methylated fatty acid standard (mixture 463, Nu-Chek-Prep, Inc., Elysian, MN, USA) was used to identify the fatty acids present and was measured and expressed as percent of total content of fatty acids based on standard curve of fatty acid.

Biological properties of Jameed (antioxidant activity, ACE inhibition activity, α -amylase inhibition)

Antioxidant activity, ACE inhibition activity, and α -amylase inhibition of Jameed extract were determined according to the method previously described by Alu'datt et al. (2012).

Experimental design and data analysis

Jameed from each treatment was prepared in duplicate. Data were analyzed using the Proc GLM procedure of SAS (SAS 2002, Institute Inc., Cary, NC, USA) as a complete randomized design. The following model was used for variables

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Y_{jk}	The dependent variable
μ	over all mean
α_i	effect of drying method
β_j	effect of salt addition
$\alpha\beta_{ij}$	interaction effect (influence of drying method and presence of salt)
ε_{ij}	The residual error

Interaction means were reported and were separated using the PDIF option ($P<0.05$) of the LSMEANS statement in PROC GLM.

Results and discussion

Effect of drying technique and salt treatment on molecular characterization of protein subunits for jameed prepared from goat milk by SDS-PAGE

Data in Fig. 2 and Table 1 showed the SDS-PAGE for Jameed prepared using different preparation techniques. The protein

Fig. 2 SDS-PAGE for prepared Jameed by sun drying (Sd) and freeze drying (Fd) techniques using salt (Sa) and unsalted (Us) treatments from goat milk; 1 SdSa; 2 FdSa; 3 SdUs; 4 FdUs

subunits for prepared Jameed by freeze drying with or without salt were similar to those of sun dried Jameed. SDS-PAGE showed that sun and freeze drying Jameed had little effect on the electropherogram of protein subunits. Whereas the effect of using salt in preparation of Jameed was more pronounced. New protein subunits were present in both of sun and freeze dried unsalted Jameed compared to salted Jameed. Results revealed that the addition of salt during decreased the growth of hydrolytic bacteria, mold and yeast microorganisms. The new protein subunits found in unsalted Jameed were due to the protein hydrolytic activities of bacteria, mold and yeast. The new protein subunits in prepared Jameed by FdUs and SdUs enhance the nutritional quality as compared to prepared Jameed by FdSa and SdSa.

Effect of salting and drying techniques on colour values of prepared Jameed from goat milk

Data in Fig. 3 shows the lightness (L^*), redness (a^*), and yellowness (b^*) values of Jameed prepared by different methods. Results show significant variation in lightness and yellowness for Jameed prepared by all four methods. However, the treatment effect was less pronounced in redness. Jameed prepared by different treatments varied significantly in L^* value. Lightness value is ranked, from highest to lowest: FdSa, SdSa, FdUs and SdUs. No research has been conducted before to evaluate the influence of salt addition and drying method on the color of prepared Jameed. However, these results suggest that salt addition has a greater influence on

Table 1 Estimated Molecular weight (MW) of protein subunits (Da) using SDS-PAGE for Jameed prepared from goat milk by sun drying (Sd) and freeze drying (Fd) techniques with salt (Sa) and without salt (Us)

Protein subunits	MW of SdSa	MW of FdSa	MW of SdUs	MW of FdUs	Literature value of milk
α -lactoalbumine	12,200	12,200	12,200	12,200	12,680
New Band	ND	ND	14,845	13,560	—
β -lactoglobulin	16,678	16,678	15,675	15,255	19,962
κ -casein	ND	ND	ND	23,417	21,428
β -casein	26,625	26,625	26,625	26,625	24,849
α S1 -casein	31,200	31,200	31,200	31,200	26,372
α S2 -casein	31,200	31,200	31,200	31,200	26,372
Ig heavy chain	50,250	50,250	50,250	50,250	53,600
Serum albumin	53,815	53,815	53,815	53,815	66,313
Lactoferrin	69,875	69,875	69,875	69,875	77,399
Butyrophilin	85,800	85,800	85,800	85,800	59,228
Xanthine dehydrogenase	134,000	134,000	134,000	134,000	146,978

Fig. 3 Color values of lightness (L^*), redness (a^*) and yellowness (b^*) for Jameed prepared by sun drying (Sd) and freeze drying (Fd) techniques using salt (Sa) and without salt (Us) treatments from goat milk. Means with different letters for the color measurement among FdSa, SdSa, FdUs and SdUs are significantly different

lightness than does drying techniques. The salt influence on Jameed lightness could be attributed to two factors: firstly, salt addition may increase light reflection and this might increase L^* values. Secondly, presence of salt in preparation of Jameed may inhibit the growth of hydrolytic microorganisms and enhance the lightness of Jameed. However, bacterial count would be required to confirm these results. Both factors have an influence of chemical composition of Jameed which has been reported to influence lightness of dairy products (Othman et al. 2012). Jameed preparation method significantly affected redness/greenness values as they ranked from highest to lowest, SdSa, FdUs, FdSa, and SdUs. Yellowness/blueness ranked from highest to lowest FdUs, SdSa, SdUs, and FdSa. However and from Jordanian consumer's quality prospective, increasing the lightness is a key factor to enhancing the color quality of Jameed.

The lightness values varied significantly among prepared by SdSa, FdSa, SdUs and FdUs. The highest L^* value was found in prepared Jameed prepared by FdSa with value of 88.02, while the lowest was found in that prepared by SdUs with value of 76.20. The maximum L^* values were obtained by preparing Jameed using FdSa. The values of redness for prepared Jameed by SdSa, FdSa, SdUs and FdUs varied significantly. The highest redness value of redness was obtained in Jameed prepared by SdSa with value -3.08 . While the lowest redness value of found in Jameed prepared by SdUs with value of -2.36 . The highest yellowness values were found in Jameed prepared by FdUs with value of 26.46. However the lowest b^* values were found in prepared Jameed by FdSa with value of 20.44. Obtained results revealed that the Jameed prepared by SdUs had a lower redness value as compared to other treatments. While that prepared by FdSa had a lower b^* value as compared to other treatments. Preparation Jameed by FdSa from goat milk may be recommended to be used potential commercial application in food industry due to high degree of lightness and lower value of

redness and yellowness which is a main significant factors in the quality of final product.

Effect of salting and drying techniques on fatty acid profile and concentration

Data in Tables 2 and 3 show the fatty acids profile and composition, respectively, of Jameed prepared using different techniques. The fatty acid profile and composition varied significantly between processes. While levels of fatty acids varied between treatments, there were a few instances where the differences were large. For example, Palmitoleic acid concentration in Jameed prepared by FdSa was almost five times (1.54/100 g fat) that compared to Jameed prepared by SdUs (0.3/100 g fat). The presence of large amounts of free fatty acids have been reported to facilitate the rate of lipid oxidation, and enhancing free fatty acids oxidation slightly more rapidly than those esterified to glycerol (Nouira et al. 2011). Even-numbered fatty acids from butyric to lauric are considered to be the major contributors to rancid flavor (Park 2001; Ha and Lindsay 1991). Thus and according to current findings, Jameed prepared by SdSa can be expected to have rancid flavor compared to Jameed prepared by other treatments due to higher concentration of even-numbered fatty acids from butyric to lauric acids. The development of rancid

Table 2 Fatty acids concentrations (g/100 g fat) of Jameed prepared by sun drying (Sd) and freeze drying (Fd) techniques using salt (Sa) and without salt (Us) treatments from goat milk GC^(a)

Fatty acids	Formula	Jameed from goat milk				
		FdSa	SdSa	FdUs	SdUs	SE
Butyric	C 4:0	5.47 ^a	5.49 ^a	4.67 ^b	3.37 ^c	0.038
Caproic	C 6:0	16.36 ^a	16.70 ^a	14.87 ^b	11.53 ^c	0.192
Caprylic	C 8:0	0.97 ^a	0.96 ^a	0.89 ^a	0.68 ^b	0.025
Capric	C 10:0	7.17 ^{ab}	7.51 ^a	6.90 ^b	5.87 ^c	0.114
Lauric	C 12:0	0.45 ^b	0.47 ^a	0.44 ^b	0.39 ^c	0.004
Myristic	C 14:0	11.77 ^{ab}	12.19 ^a	11.69 ^{ab}	11.24 ^b	0.200
Palmitic	C 16:0	27.56 ^b	28.90 ^b	28.76 ^b	31.51 ^a	0.567
Palmitoleic	C 16:1	1.54 ^a	0.76 ^{ab}	1.53 ^a	0.30 ^b	0.281
Margaric	C 17:0	0.79 ^a	0.71 ^a	0.79 ^a	0.96 ^a	0.117
Heptadecenoic	C 17:1	0.36 ^b	0.32 ^b	0.35 ^b	0.44 ^a	0.013
Stearic	C 18:0	5.56 ^b	5.88 ^b	5.95 ^b	7.37 ^a	0.121
Oleic	C 18:1n9t	2.31 ^a	1.84 ^a	2.64 ^a	2.76 ^a	0.347
Oleic	C 18:1n9c	16.01 ^b	15.13 ^b	16.65 ^b	20.35 ^a	0.589
Linoleic	C 18:2	2.76 ^a	2.36 ^a	2.88 ^a	2.56 ^a	0.091
Linolenic	C 18:3	0.44 ^a	0.36 ^b	0.44 ^a	0.41 ^{ab}	0.014
Arachidic	C 20:0	0.53 ^b	0.44 ^c	0.57 ^a	0.31 ^d	0.012

^(a) Means are average of two replicates

*Means with different letters in the same row are significantly different at $P \leq 0.05$

Table 3 Fatty acid composition (g/100 g fat) of Jameed prepared by sun drying (Sd) and freeze drying (Fd) techniques using salt (Sa) and without salt (Us) treatments from goat milk

Fatty acids	Jameed from goat milk ^(a)				
	FdSa	SdSa	FdUs	SdUs	SE
Short Chain (C 4:0 - C 6:0)	21.82 ^a	22.19 ^a	19.54 ^b	14.90 ^c	0.222
Medium Chain (C 8:0- C 14:0)	8.14 ^{ab}	8.47 ^a	7.79 ^c	6.54 ^b	0.105
Long Chain (C 14:0- C 22:0)	70.05 ^c	69.35 ^c	72.68 ^b	78.56 ^a	0.316
Saturated	76.58 ^{ab}	79.24 ^a	75.52 ^{ab}	73.20 ^b	1.037
Unsaturated	23.42 ^{ab}	20.77 ^b	24.48 ^{ab}	26.80 ^a	1.037
Poly Unsaturated (C18:1- C18:3)	3.20 ^{ab}	2.73 ^b	3.32 ^a	2.97 ^{ab}	0.100
Even Saturated (C12:1- C18:0)	45.32 ^b	47.44 ^{ab}	46.85 ^b	50.50 ^a	0.891

*Means with different letters in the same row are significantly different at $P \leq 0.05$

flavor is caused by microbial lipolysis by microbes that contaminate dairy products and produce lipase which develops rancid flavor (Velez et al. 2010). The free fatty acid content that accumulates during ripening or drying in dairy product been attributed primarily to lipolysis. Manufacturing method is considered one of main factors that affect the rate of lipolysis (Velez et al. 2010; Collins et al. 2003).

Data in Table 2 shows the influence of different processing treatments on fatty acid composition of Jameed. Different fatty acid concentrations due to processing treatment may suggest different nutritional and sensory characteristics (Nouira et al. 2011). Previous studies have shown that biochemical and physical modifications can occur in dairy products made from goat milk after manufacture due to ripening and degradation of nutrients in the products (Fox 1989; Park 2001). One of the important changes is that reported in polyunsaturated fatty acids (PUFA). This is an important change because their favourable effect on the health of humans, making them the most valuable group among other fatty acids (Rodriguez-Alcala et al. 2009). In this study, Jameed prepared by SdSa had the lowest polyunsaturated fatty acid content and highest saturated fatty acid concentration among treatments.

Effect of salting and drying techniques on therapeutic properties of prepared Jameed from goat milk

Data in Table 4 illustrates the antioxidant, ACE inhibitory and α -amylase inhibitory activities of Jameed prepared by SdSa, FdSa, SdUs and FdUs. Although salt addition resulted in a relatively high antioxidant activity, the antioxidant activities for that prepared by FdSa was lower (59.57 %) compared to Jameed prepared by SdSa (64.53 %). Without salt addition, Jameed prepared by freeze drying (i.e. FdUs) maintained a higher antioxidant activity (49.54 %) compared to Jameed

Table 4 Antioxidant (%), ACE inhibitory activity (%) and α -amylase inhibitory activity (%) of Jameed prepared by sun drying (Sd) and freeze drying (Fd) techniques using salt (Sa) and without salt (Us) treatments goat milk

Treatment	Antioxidant activity (%)	ACE inhibitory activity (%)	α -amylase inhibitory activity (%)
FdSa	59.57 ^b	27.90 ^a	67.26 ^c
SdSa	64.53 ^a	11.74 ^c	89.58 ^a
FdUs	49.54 ^c	19.13 ^b	76.19 ^{bc}
SdUs	40.80 ^d	29.13 ^a	83.63 ^{ab}
SE	1.26	0.54	2.35

^a Means are average of three replicates

*Mean values with the same superscript in a column are not significantly different ($p > 0.05$)

prepared by sun drying (i.e. SdUs) (40.80 %). As expected, these results revealed that the using salt in preparation of Jameed increases the shelf life possibly by reducing microbial growth. Milk is known to contain several factors (vitamins, enzymes and peptides) that are reported to have antioxidative activities (Pihlanto 2006). However, addition of salt has been reported to decrease proteolysis and lipolysis of harmful bacteria (El-Bakry 2012) which might help Jameed to maintain a higher antioxidant activity. However, no beneficial influence occurred due to combining salt treatment and freeze drying (i.e. FdSa). Distribution of salt while freeze drying may influenced bacterial growth within the Jameed as rapid water evaporation may cause uneven distribution of salt components within Jameed.

ACE is an enzyme that has a key role in the rennin-angiotensin system, which in turn regulates the arterial blood pressure and the equilibrium of water and salt in the body (FitzGerald et al. 2004). Many peptides in milk have been reported to possess antihypertensive action upon fermentation of milk with different microorganisms, or by the action of pure proteinases on milk proteins (Papadimitriou et al. 2007). However and to the best of our knowledge, no research had been conducted to study the influence of Jameed prepared from goat milk on ACE activity. In this experiment, the inhibitory activity of ACE for Jameed prepared by FdSa or SdUs varied significantly as compared other treatments. The maximum inhibitory activities of ACE occurred in Jameed prepared using SdUs (29.13 %) and FdSa (27.90 %), lowest for that prepared using SdSa (11.74 %) and intermediate for FdUs (19.13 %). Although there were differences between treatments, results revealed that preparation of Jameed with or without salt may enhance the inhibitory activities of ACE.

Table 4 shows that sun drying has a greater influence on amylase inhibitory activity than does the freeze drying method. The highest inhibitory activities of α -amylase were obtained in Jameed prepared by SdSa and SdUs with values of 89.58 and 83.63 %, respectively. Lower inhibitory activities of

α -amylase were obtained in Jameed prepared by FdUs and FdSa (76.19 and 67.26 %, respectively). These results indicate that Jameed possesses inhibitory activity on α -amylase and the drying method has greater influence.

Conclusion

It can be concluded that freeze drying with or without salt can be an alternative approach to the sun drying method to process Jameed from goat milk. Different processing treatments of Jameed can alter the fatty acid profile and concentration and thus change nutritional and physical properties. The best color values were obtained in Jameed prepared by FdSa. The short chain fatty acid content increased in Jameed prepared by sun drying compared to that prepared by freeze drying. The preparation of Jameed by SdUs decreased the content of caprylic acid. Addition of salt in conjunction with the drying method (sun drying vs freeze drying) influenced biological activities (antioxidant activity, and inhibitory activities of ACE and α -amylase) of Jameed as well.

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