ELSEVIER

Food Chemistry 80 (2003) 85–89

Food Chemistry

www.elsevier.com/locate/foodchem

Evaluation of (*E*)-10-hydroxydec-2-enoic acid as a freshness parameter for royal jelly

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Received 4 September 2001; received in revised form 22 May 2002; accepted 22 May 2002

Abstract

(*E*)-10-hydroxydec-2-enoic acid (10-HDA) is the main fatty acid present in royal jelly (RJ). Among the criteria for royal jelly quality analysis, 10-HDA content has been proposed as a freshness parameter. In this study, 10-HDA content variations under controlled temperature storage, were evaluated as an aging indicator of RJ samples from two origins (France and Thailand). For a 12 month period, the 10-HDA content loss rates were 0.1 and 0.2% at −18 °C and 4 °C, respectively, whatever the sample origin. At room temperature storage, losses were 0.4 and 0.6% for the French sample and the Thaï sample, respectively. No correlation was found between 10-HDA content and storage duration whatever the storage temperature. Even if 10-HDA is specific to RJ, new criteria should be found to estimate the condition and duration of storage for royal jelly samples.

Keywords: (*E*)-10-hydroxy-decen-2-oic acid; Quality; Royal jelly; *Apis mellifera*

1. Introduction

Royal jelly is a secretion from the hypo-pharyngeal and mandibular glands of worker bees (*Apis mellifera*) in relation to sexual determination of the bee (Goewie, 1978). Considered as the major cause for difference between queen and bee workers, royal jelly (RJ) is appreciated as a dietary complement because of its composition. For economic reasons and concerns for human health, the composition of RJ has been extensively studied in order to find appropriate composition criteria.

Methods developed to characterize royal jelly consist in determining general parameters such as water content, sugars (Howe, Dimick, & Benton, 1985; Lercker, Savioli, & Vechi, 1986; Palma, 1992; Serra Bonvehi, 1992; Tourn, Lombard, Belliardo, & Buffa, 1980; Takenata, 1982), lipids (Lercker et al., 1992–1993; Lercker, Capella, & Conte, 1981, 1982) and proteins (Hanes & Simuth, 1992; Serra Bonhevi, 1990; Thrasyvoulou, Collison, & Benton, 1983) by using high pressure liquid chromatography, gas chromatography or SDS-PAGE analysis.

These studies have shown the predominance of hydroxy acids in the lipidic fraction of RJ. These functionalized acids are implicated in the regulation of the activities of the hive, and in metabolic pathways, some of which are specific to the queen (Plettner, Otis, Wimalaratine, & Punchihewa, 1997; Plettner, Slessor, Winston, & Oliver, 1996; Winston, 1987). Their effect in human nutrition is not well known, partly due to the small number of studies.

Of particular interest has been the occurrence, in the lipidic fraction, of (*E*)-10-hydroxydec-2-enoic acid (10-HDA), which appears to be a fatty acid specific to the RJ (Baker, Foster, Lamb, & Hodgson, 1959). Thus the 10-HDA content has been adopted as a marker for RJ and is currently used as a means to evaluate bee products containing RJ (Bloodworth, Harn, & Hock, 1992).

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0308-8146/02/$ - see front matter
PII: S0308-8146(02)00243-1

Sugar composition, humidity, protein and lipid contents are the most common criteria used for characterizing RJ quality (Pourtallier, Davico, & Rognone 1990). More recently 10-HDA content tends to be accepted by beekeepers and manufacturers as a freshness indicator: royal jellies with 10-HDA content greater than 1.8% have been then considered to be fresh and authentic samples. However, the variation of 10-HDA according to the storage conditions remaines poorly investigated.

The objective of this study was to further evaluate the variation of the 10-HDA content under controlled storage conditions. For this purpose, 10-HDA content of samples stored at −18 °C, 4 °C and room temperature were investigated during 12 months. This approach was chosen with an aim to evaluate 10-HDA content as a representative of short storage duration, as is necessary for the labelling of fresh products.

A complementary study was conducted to evaluate the effect of repeated temperature variation and homogenization on 10-HDA content under standard storage conditions.

2. Materials and methods

2.1. Materials

Two freshly harvested royal jelly samples were obtained from France and Thailand. These samples were collected in September 1998 and stored at 4 °C for 5 days before analysis. Fifteen fresh samples of various origins were also purchased from beekeepers. Standard of (*E*)-10-hydroxydec-2-enoic acid (10-HDA) was purchased from Nippon Shoji Kaisha compagny (Osaka, Japan).

2.2. Methods

2.2.1. Experimental protocol

In order to evaluate the variation due to the storage temperature while minimizing any other influencing factor, both samples were left at room temperature for one hour, then homogenized and divided into aliquots (6 g). These vials were protected from light with aluminium foil wrapping. Each group of aliquots was stored at −18 °C, 4 °C and room temperature (25° ± 2 °C), respectively. Each month, one aliquot of each definite storage condition was analysed in duplicate (S_{month} value). Cold samples were equilibrated at room temperature for 1 hour and then homogenized before analysis.

In order to evaluate the effect of repeated temperature variations and homogenization, according to common commercial practice, for each geographical origin, two bulk aliquots (40 g) were also kept under the above mentioned cold conditions. These aliquots were analyzed every month (R_{month} value). Prior to analysis, these aliquots were equilibrated at room temperature for one hour and homogenized. The experimental protocol is summarized in Fig. 1.

Additionally, 15 other samples, of various origins, were stored at 4 °C during 15 months and analyzed before and at the end of this storage duration.

2.2.2. Sample preparation

In a typical analysis, a weighed mass of about 2.5 g of RJ was dissolved by sonication in 100 ml of a methanol solution (50:50 v/v with ultrapure, deionized and CO_2-free water) adjusted at pH = 2.5 with phosphoric acid.

2.2.3. High performance liquid chromatography

The 10-HDA content of RJ was determined by high performance liquid chromatography with diode array detection (Bloodworth et al., 1995). Twenty µl of the sample solution, previously diluted 10 times and filtered, were eluted on a RP-8 column (Lichrospher 100, 5 µm, 250×4 mm) with an aqueous solution of methanol as eluant. The analysis was performed on an Hewlett Packard 1050 liquid chromatograph, connected to an diode array detector (210 nm, reference value 360 nm). Quantitative determination of 10-HDA was achieved by using a calibration curve based on five standard solutions of pure 10-HDA.

2.2.4. Calculation

Prior to this study, the repeatability, reproductibility and detection limit of the method were established, as published elsewhere (AFNOR V03-110, 1993; Lynch, 1998). The limit of detection for 10-HDA and the confidence interval were 0.064% and 0.08, respectively.

By convention, 10-HDA content (%) was expressed as the ratio of the amount of 10-HDA detected to the amount of royal jelly weighed.

3. Results and discussion

3.1. Influence of the storage temperature

A rapid degradation of the samples stored at room temperature appeared after the third month. This degradation was characterized by the development of an acreous odour and the separation into two layers after the sixth month. The storage at room temperature was previously correlated with an increase in viscosity and the development of browning-Maillard reactions (Chen & Chen, 1995; Karaali, Moydanoglu, & Eke, 1998), which appeared minimal at cold temperatures. No significant difference was observed, by sensory evaluation, for the aliquots stored at low temperature throughout the study.

Fig. 2 shows a typical chromatogram allowing the accurate quantification of 10-HDA in RJ. Observed

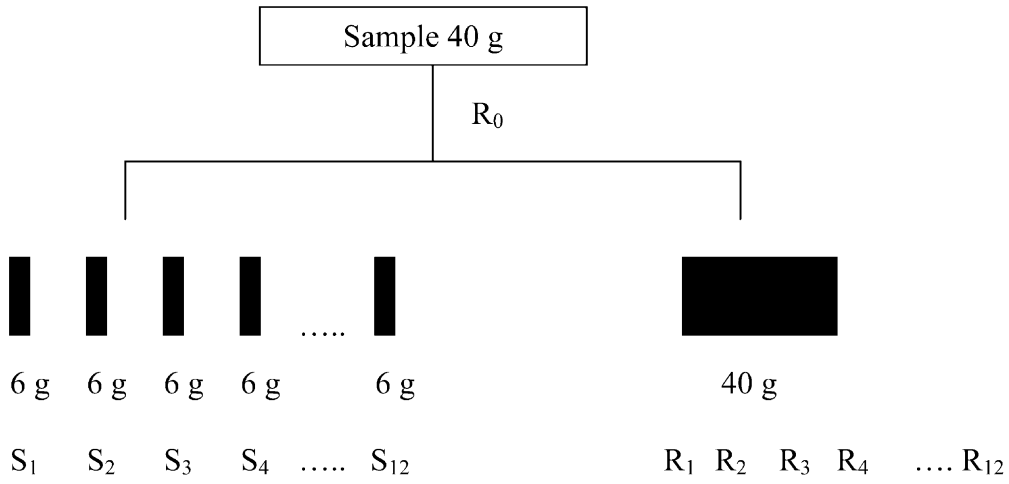

Fig. 1. Experimental Protocol. S and R values were measured for each storage condition (room temperature, +4 °C, −18 °C).

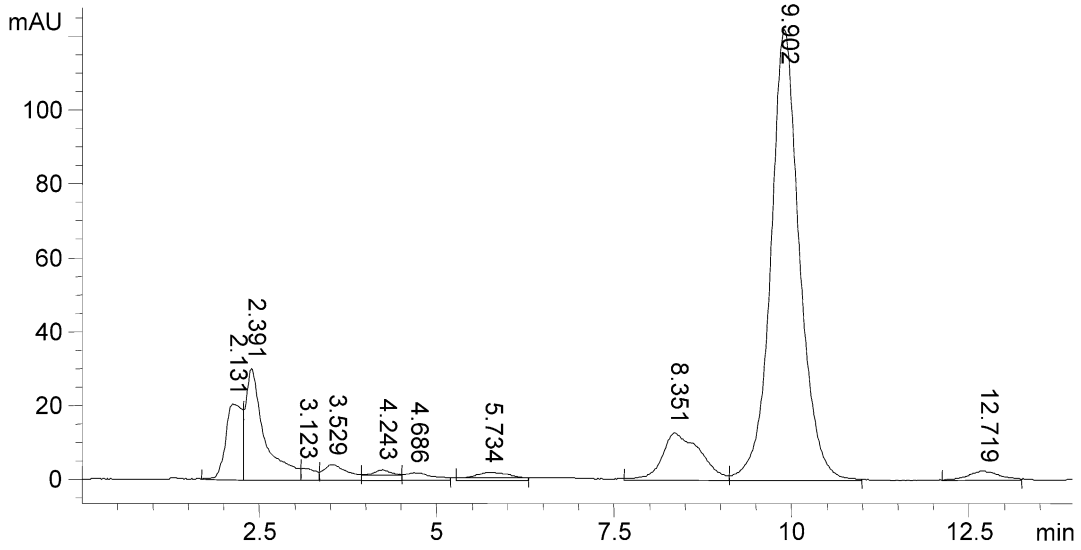

Fig. 2. Typical chromatogram for the quantification of (10-HDA) in royal jelly. Retention time of 10-HDA = 9.9 minute. Signal acquisition at 210.4 nm with a reference value at 360 nm.

differences between duplicates were lower than 0.1, as etablished by the validation issue of the methodology.

The overall comparison showed variation reaching 0.6% (from 2.6 to 2.0%) and 0.4% (from 1.8 to 1.4%) for the French and Thaï samples stored at room temperature (Fig. 3). From these values, a relative percentage loss of 30 and 28.5% could be calculated. However, no particular variation on the curve showed any correspondence with physical degradation after the third month. Loss of 10-HDA appeared more distinct after the sixth month.

Presumably the large difference in 10-HDA content measured for the two samples should not be considered as characteristic of the geographic origin. Values ranging from 1.7 to 2.4% are commonly observed, whatever the origin of RJ.

Table 1 summarizes the R_{month} and S_{month} values obtained for lower temperatures. Under cold conditions, the observed variation was significantly decreased. Whatever the geographic origin of the product, the overall variation (S_{month}) was similar: 0.2% (French sample) and 0.3% (Thaï sample) at 4 °C and 0.1% at −18 °C. According to these data, cold storage conditions appeared to minimize the decrease in 10-HDA content. Relative percentage losses associated with these variations are 8.3% (French sample, 4 °C), 20% (Thaï sample, 4 °C), 4% (French sample, −18 °C) and 5.9% (Thaï sample, −18 °C). These losses appeared more pronounced at 4 °C and non-significant at −18 °C taking into account the performance statistics of the quantification. Under cold conditions, 10-HDA content did not significantly decrease during storage. In practice, RJ is considered as fresh when less than 3 month's old. For this purpose, absolute 10-HDA content is not appropriate because cold temperature decreases the 10-HDA degradation of 10-HDA for long periods of

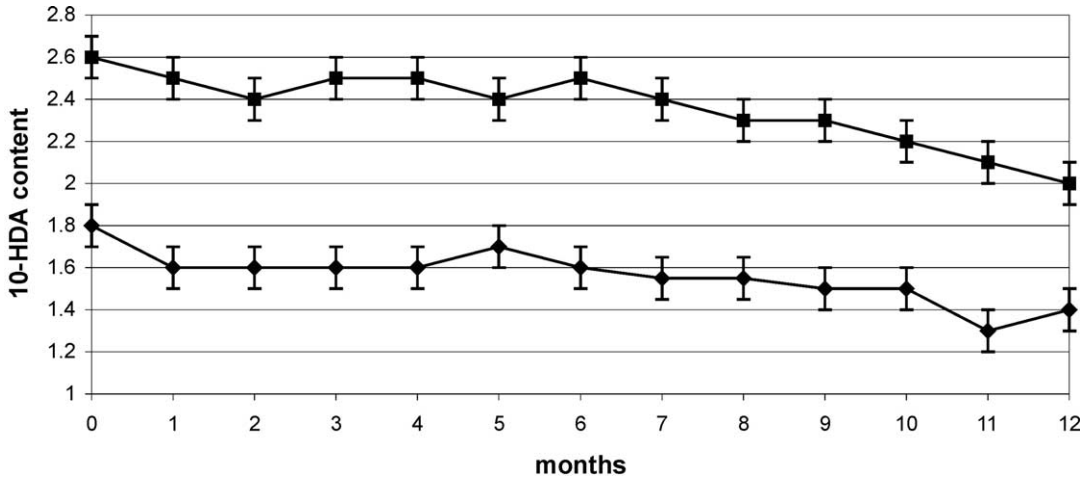

Fig. 3. Variation of 10-HDA content (% of fresh matter) according to the storage duration at room temperature. (◆) Thaï sample; (■) French sample. Error bars show the incertitude related to the quantification (value = 0.1).

Table 1
Variation of 10-HDA content according to cold storage condition

Month	French				Thaï			
	R_{month}		S_{month}		R_{month}		S_{month}	
	−18 °C	+4 °C	−18 °C	+4 °C	−18 °C	+4 °C	−18 °C	4 °C
1	2.6	2.6	2.6	2.6	1.8	1.8	1.8	1.8
2	2.5	2.5	2.5	2.5	1.8	1.7	1.8	1.7
3	2.6	2.5	2.6	2.5	1.7	1.7	1.7	1.7
4	2.5	2.5	2.6	2.5	1.7	1.6	1.7	1.6
5	2.5	2.4	2.5	2.4	1.8	1.5	1.8	1.7
6	2.6	2.4	2.6	2.5	1.8	1.3	1.7	1.6
7	2.6	2.4	2.5	2.5	1.7	1.4	1.8	1.6
8	2.5	2.4	2.6	2.5	1.7	1.3	1.7	1.6
9	2.5	2.4	2.6	2.5	1.8	1.3	1.7	1.6
10	2.5	2.3	2.5	2.4	1.7	1.3	1.7	1.6
11	2.5	2.3	2.5	2.4	1.7	1.2	1.8	1.6
12	2.5	2.2	2.5	2.4	1.7	1.2	1.7	1.5

Mean value of duplicates expressed in % of 10-HDA. Initial values (R_0) were, respectively, 2.6 and 1.8% for French and Thaï samples.

time. Moreover, the degradation rate is very low when RJ is stored at room temperature; the determination of 10-HDA does not allow us to characterize a short storage period at room temperature.

The analysis of the 15 supplementary samples stored at 4 °C for 15 months showed an overall decrease of 10-HDA content ranging between 0.1 and 0.2% (data not shown). This last result confirms that 10-HDA content could relate to long periods of storage under cold conditions. As a consequence, whereas 10-HDA is specific to RJ, the measure of 10-HDA content cannot be considered as a freshness criterion.

3.2. Influence of the homogeneization and short variations of temperature

When stored at −18 °C, no significant 10-HDA variation was observed, whatever the origin of the sample or the protocol. However, at 4 °C, the relative losses were 20 and 50% for the French and the Thaï sample, respectively. Although the same protocol was applied to both samples, the origin of the difference remains unclear.

Despite the small number of samples used in this evaluation, the combined homogenization and repeated short variations of temperature tend to increase the loss of 10-HDA during long storage at 4 °C. A lipidic oxidation, due to the incorporation of air associated with an enzymatic pathway active at 4 °C, is probably responsible for 10-HDA loss. However, to our knowledge, no study deals with the stability of unsaturated hydroxy acids and related oxidation reactions in the food matrix. The degradation of 10-HDA seems to be stopped at −18 °C. Among the factors affecting the decrease of 10 HDA at 4 °C, oxidation seems more important than storage duration.

In a similar way, significant variations in the water-soluble protein fraction and sugars (glucose, fructose, saccharose) were previously reported for storage at 4 °C (Chen & Chen, 1995). These results could be very important in beekeeping practice. Under standard conditions of production, RJ is collected in a glass recipient with a capacity ranging from 100 to 1000 g, then stored at 4 °C. For selling purposes, this sample is equilibrated at room temperature, homogenized and the required quantity conditioned. The remaining sample is then stored again at low temperature. Thus, the protocol involved is similar to those applied for the determination of R_{month} value and could be non negligible in the evolution of the 10-HDA content over the period of storage. A suitable volume of storage for commercial purposes should be adequate to decrease these variations.

A new physico-chemical parameter is required to characterize the freshness and storage duration of RJ. The determination of coloration, viscosity and water-soluble protein fraction were proposed by Chen and Chen (1995)

in order to evaluate the change in quality during the storage. However, due to the natural variability observed in commercial RJ samples, these criteria are not easily standardized. As a result, no freshness criteria seem currently available for RJ. Further studies are necessary to elucidate the effects of beekeeping practice and the influence of climatic and storage conditions on RJ composition. These studies are prerequisites for establishing quality criteria for RJ.

Acknowledgements

We thank Dr. Michel Pépin and Dr. Michel Aubert for their critical review of the manuscript.

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