Characterization of Rosemary Essential Oil for biodegradable emulsions

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1   **Abstract**

2   The characterization of rosemary essential oil (EO) for its formulation in biodegradable

3   emulsions has been carried out. Firstly, the required HLB (hydrophile–lipophile balance) value

4   of the oil was determined to be 15 based on droplet size analysis and the stability of emulsions

5   with synthetic surfactants. Moreover the emulsion resulted to be stable after 50 days of

6   storage in ambient conditions. Secondly, four biodegradable and non-toxic surfactants derived

7   from starch were tested. The effect of these surfactants was analyzed by measuring interfacial

8   tension between the oil and the aqueous phase.

9   **Keywords**: Essential oil, emulsion, required HLB, surface tension, surfactant

10   **1. Introduction**

11   Essential oils (EOs) are commonly used as natural preservatives and fragrances in cosmetic

12   products. More recently, thanks mainly to their antimicrobial properties, new applications as

13   food preservatives (Burt, 2004), growth promoters in livestock (Calsamiglia et al., 2007),

14   natural pesticides in organic agriculture (El-Shafei et al., 2010) and insecticides (Phillips et al.,

15   2010) are emerging. EOs typically are volatile and they rapidly evaporate from surfaces. It is

16   thus desirable to formulate them, in its effective concentration, in a way that allows

17   minimizing the evaporation and protecting the oil, from high temperature, oxidation and UV

18   light, at the same time. Besides, such formulations should allow for a selective release and for

19   the increase of the shelf life of the oil. For this purpose, oil-in-water (O/W) emulsions are one

20   of the preferred formulations of EOs.

21 Stable emulsions are best formulated with emulsifiers or combination of emulsifiers having

22 HLB (hydrophile–lipophile balance) values close to that required for the oil phase (Aulton,

23 1995). The required HLB values of commonly used fixed and mineral oils have been

24 determined but those of EOs are yet to be studied.

25 The HLB of rosemary EO has been determined according to the method proposed and

26 validated by Orafidiya and Oladimeji (2002) based on the determination of droplet size and

27 size distribution of emulsions prepared with surfactants of a wide range of HLB values, being

28 the required HLB for the essential oil that of the surfactant leading to the most stable

29 emulsion, i.e. that of the minimum droplet size.

30 As previously mentioned, this required HLB value of the oil phase will be useful to select an

31 appropriate surfactant for the emulsion. These data is mainly available for synthetic

32 surfactants or it can be calculated from the chemical formula by group contribution methods

33 (Tadros, 2005; Wang et al., 2011). However, this information is not always available for

34 natural or natural derived surfactants, such as n-octenyl succinic anhydride (OSA)-modified

35 starches, whose application in emulsion formulation is increasing in the last years (Varona et

36 al., 2009; Wang et al., 2011).

37 In such cases, the measurement of the interfacial tension between the oil and a water solution

38 of the surfactant whose HLB value is unknown, provide reliable and fast information for the

39 selection of the appropriate surfactant and the minimum concentration needed to obtain a

40 stable emulsion (Huang et al., 2011) which is generally higher than the critical micelle

41 concentration (CMC) for the corresponding surfactant, provided that this information is

42 available. The smaller the interfacial tension between the oil phase and water, the more stable

43 will be the emulsion as it adsorbs onto the oil forming interfacial film, reducing the interactions

44 between both phases and the energy required to expand the interface of the phase to be

45 dispersed, in this case the oily one, for the formation of the emulsion (Tadros, 2005).

46   Hence, in the second part of this work the interfacial tension between the Rosemary EO and

47   the water for four biodegradable and non-toxic surfactants, n-octenyl succinic anhydride

48   (OSA)- modified starches, was measured and compared with the interfacial tension of the EO

49   and a synthetic surfactant with a value of HLB equal to that required HLB by the EO, in order to

50   select the most adequate OSA-starch and the minimum concentration required to obtain a

51   stable emulsion.

52   **2. Materials and Methods**

53   2.1. Materials

54   The essential rosemary (*Rosmarinus officinalis L.*) oil was supplied by COCOPE (Peñafiel, Spain).

55   It was obtained by steam distillation. The surfactants, Span® 20,Tween® 20 and Tween® 80,

56   were purchased from Sigma (Spain). The (OSA)-modified starches were kindly provided by

57   National Starch Food Innovation (Hamburg, Germany). The followings were tested: OSA-starch

58   derived from waxy maize (OSA-1), OSA-starch derived from waxy maize blend with dried

59   glucose syrup (OSA-2), OSA-dextrin derived from waxy maize (OSA-3) and OSA-dextrin derived

60   from tapioca (OSA-4).

61   The main components of the rosemary oil analyzed by Gas Chromatography are displayed in

62   Table 1.

63   2.2. Methods

64   *2.2.1. Preparation of the emulsion*

65   Oil-in-water emulsions were prepared by a two-step process. A surfactant solution (200 mL)

66   was initially prepared by dissolving the surfactant at a concentration of 1g/L in deionized water

67   (Milli-Q, Millipore) with the aid of a magnetic stirrer (IKA). Afterwards 50 mL essential oil was

68   gradually added to the suspension under continuous agitation for 5 min and a crude emulsion

69   was obtained. The resulting coarsely dispersed raw emulsion was then fed into the rotor-stator

70 machine (IKA® LABOR PILOT 2000/4) and processed during 2 min for fine emulsification at

71 70Hz, according to Varona et al, 2009.

72 *2.2.2. Preparation of the mixture of surfactants*

73 Emulsions were prepared using a combination of two surfactants, Span 20 whose HLB is 8.6

74 and Tween 20 whose HLB is 16.7. The proportion between these two surfactants was changed

75 in order to obtain HLB values in-between. The HLB of the mixture was calculated according to:

76 $HLB = x_A \cdot HLB_A + x_B \cdot HLB_B$ (1)

77 where x_A and x_B are the weight fractions of each surfactant.

78 *2.2.3. Droplet size analysis*

79 Rosemary oil droplet size distribution of the emulsion was determined by laser diffraction

80 (Mastersizer 2000, Malvern Instruments) with a dual-wavelength detection system (Red light:

81 633 nm and Blue light: 436 nm). The sample was diluted with deionized water in the dispersion

82 unit (Hydro SM) to less than 1% (v/v) to prevent multiple scattering effects. The refractive

83 index of the dispersed phase, rosemary essential oil, was experimentally determined (Stanley

84 Abbey Refractometer) to be 1.468, in good agreement with literature values (Atti-Santos et al.,

85 2005). Particle size measurements are reported as the 10%, 50% and 90% quantiles of the

86 volume distribution, i.e. the 50% volume of droplets have a size smaller than the $d_{50,v}$ value;

87 The values are the average of 3 measurements. The maximum incertitude of the analysis was

88 5%, except for stored emulsions of HLB below 10.7 that reached values up to 20%.

89 In order to observe variation during the storage time, the measurements were made directly

90 after the production of the emulsion, and 21 and 50 days later following the same procedure.

91 *2.2.4. Determination of the stability of emulsions*

92 The emulsion stability, that means the ability of an emulsion to resist changes in its properties

93 with time, was analyzed using two different criteria: the amount of de-emulsified oil at two

94   time intervals (21 and 50 days) and the kinetics of sedimentation and creaming of the

95   emulsion in the following 60 minutes after preparation.

96   For both analysis, 7 mL of the emulsion were poured in a vertical glass tube with an inner

97   diameter of 13 mm (height of emulsion: 55 mm) and stored at ambient conditions.

98   The height of visible supernatant oil layer was recorded, and compared to maximum height of

99   oil (11 mm), i.e. the emulsion were completely de-emulsified; this ratio multiple by 100 was

100   the % of de-emulsionated oil.

101   Particles migration phenomena (creaming or sedimentation) induce particle volume fraction

102   changes at the extremities of the sample. The creaming and sedimentation rates were

103   followed using a Turbiscan Classic MA2000 (Formulaction, France). The stability of emulsions is

104   followed measuring the variation of %backscattering of an 860 nm near infrared beam versus

105   the height of the sample, in steps of 40 µm, every 2 min for 60 min. The backscattering

106   detector receives the light scattered backward by the sample at an angle of 45° with respect to

107   the light source. The software of the equipment determines the calculation the slopes of the

108   %backscattering curves versus time in the lower and upper part of the emulsion (Figure 2,

109   inner box) in order to evaluate the kinetics of sedimentation and creaming, respectively. These

110   rates are then used to calculate the thickness of sedimentation and creaming after a defined

111   time (i.e. 60 minutes) for each emulsion in order to compare them.

112   *2.2.5. Evaluation of modified starch surfactants*

113   In order to evaluate the performance of the modified starch surfactant, the interfacial tension

114   between the EO and the aqueous phase was measured. The concentration of surfactant in the

115   aqueous phase ranged from 0 to 100 g/L. The interfacial tension was compared with that

116   achieved with a synthetic surfactant with a HLB equal to that required by the rosemary EO.

117   Measurements were made in triplicate using the Wilhelmy plate method with a tensiometer

118   EasyDyne (Krüss, Germany). The equilibrium time required for steady state interfacial tension

119   measurements was 2 min. The maximum standard deviation was 5%.

120   **3. Experimental results and discussion**

121   <u>3.1. Required HLB</u>

122   The required HLB value for the rosemary essential oil is between 12 and 15. According to the

123   analysis of mean drop size (Figure 1), expressed as the 50% quantile ($d_{50,v}$), a defined HLB

124   cannot be identified for a clear minimum value of the droplet size.

125   The de-emulsified oil after 50 days was neither a clear criteria, as only emulsions prepared

126   with a surfactant mixture HLB value smaller than 10.7 show a measurable (> 1 mm) layer of

127   supernatant oil. Besides, the emulsion prepared with a surfactant of HLB 14 displayed a thin

128   layer of oil with a height lower than 1mm. Only, the emulsions prepared with surfactants of

129   HLB 8.6 and 9.2 were completely de-emulsified after 50 days.

130   The analysis of the backscattered light across the emulsion allows determining the height of

131   emulsion sedimentation and creaming (e) for each emulsion, to evaluate its stability in the first

132   hour after their preparation (Figure 2). In a similar way to previous criteria of stability, all the

133   emulsions prepared with mixtures of surfactants with a HLB value higher than 10.7 have

134   similar values of "e".

135   In order to point out a specific value of required HLB in the range from 12 to 15, the width of

136   the droplet size distribution (DSD) based on the 10% and 90% quantile was analyzed. As

137   shown in Figure 3, fresh emulsions prepared with surfactant mixtures with HLB between 10.7

138   and 16.7 present a very narrow DSD. As the emulsions evolved with time, the width of the DSD

139   increases; after 21 days the initial HLB range is reduced to 13 to 15, and after 50 days, the

140   narrowest DSD is the one of the emulsion prepared with a surfactant mixture of HLB equal to

141   15. Hence, it can be concluded that the required HLB of the rosemary essential oil is 15.

142 This value is slightly higher than the values of other essential oils for aromatic herbs from the

143 order of Lamiales, such as lavandin oil with a value of 13.4 (Varona et al., 2009), lippia and

144 peppermint oils with values of 12.1 and 12.3, respectively (Orafidiya and Oladimeji, 2002).

145 Although, the HLB may be related with the hydrophilicity of the main component of the

146 essential oil, this is not always the case due to the great amount of components present in the

147 oil. In the lavandin oil, the main component is the linalool (ca. 45%), which is more hydrophilic

148 than the menthone (Phillips et al., 2010), the main component in the peppermint oil (ca.45%-

149 60%). However, the main component of the rosemary oil used in this work is the 1,8-cineole

150 (ca. 24%), which is more hydrophobic than the linalool, although HLB value of rosemary oil is

151 higher than that of lavandin oil. These can be due to the presence of other minor components

152 more hydrophilic, such as camphor (ca. 16%).

153 3.2. Evaluation of modified starch surfactants

154 As shown in Figure 4, the most adequate modified starches to be employed as surfacts with

155 rosemary essential oil, are OSA-4 and OSA-2, as they deacrease the interfacial tension between

156 water and rosemary EO from 16.2 ± 0.6 mN/m to a lower value with respect to the other

157 surfactants tested. As reference, the interfacial values using a synthetic surfactant with the

158 HLB value requiered by the rosemary EO (Tween® 80, HLB =15) are also presented. The

159 minimum concentration of the surfactant that should be used to prepare stable emulsions of

160 rosemary EO is 25 g/L, which is 5 times higher than the critical micelle concentration of these

161 two surfactants, 4.5 g/L and 5.2 g/L for OSA-2 and OSA-4, respectively (Varona et al., 2009).

162 **4. Conclusions**

163 The characterization of rosemary EO regarding its formulation in emulsion has been carried

164 out. A required HLB value of 15 was determined according to the stability of emulsions in

165 terms of minimum droplet size and width of the drop size distribution over a period of 50 days

166 storage at ambient conditions. The performance of four biodegradable and non-toxic

167 surfactants derived from starch as stabilizers for rosemary essential oil emulsions in water, was

168 tested based on interfacial tension measurements. The best surfactants, OSA-2 and OSA-4,

169 reduced the interfacial tension between water and the essential oil in two thirds.

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200   octenyl succinate starch as a delivery carrier for bioactive food components. Food Chem. 126,

201   1218-1225

Tables

Table 1. Main components of the rosemary essential oil analyzed by GC

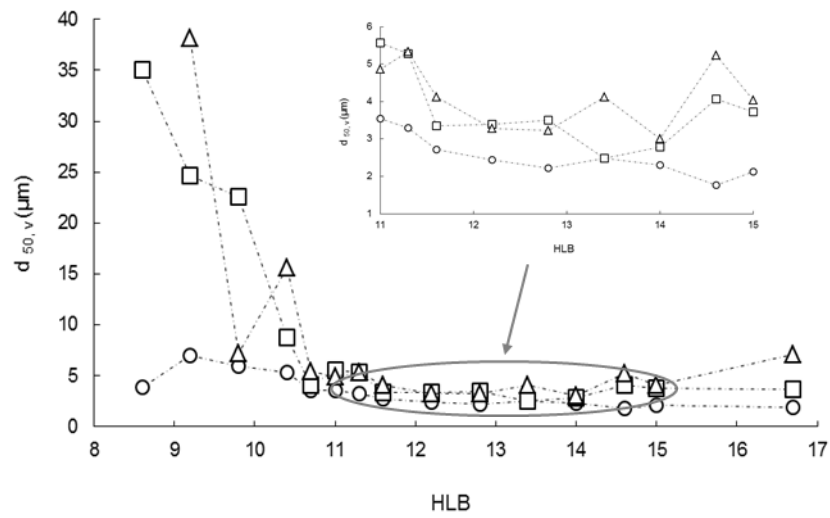| Component	wt. %
1,8-cineole	23.8
α-pinene	15.6
camphor	15.6
camphene	9.2
Bornyl acetate	5.7
β-pinene	5.1
Borneol	4.4
caryophillene	3.9
α-terpineol	1.5

Figures and captions

Figure 1. Drop emulsion diameter for different HLB values. Evolution with time: ○ - 0 days
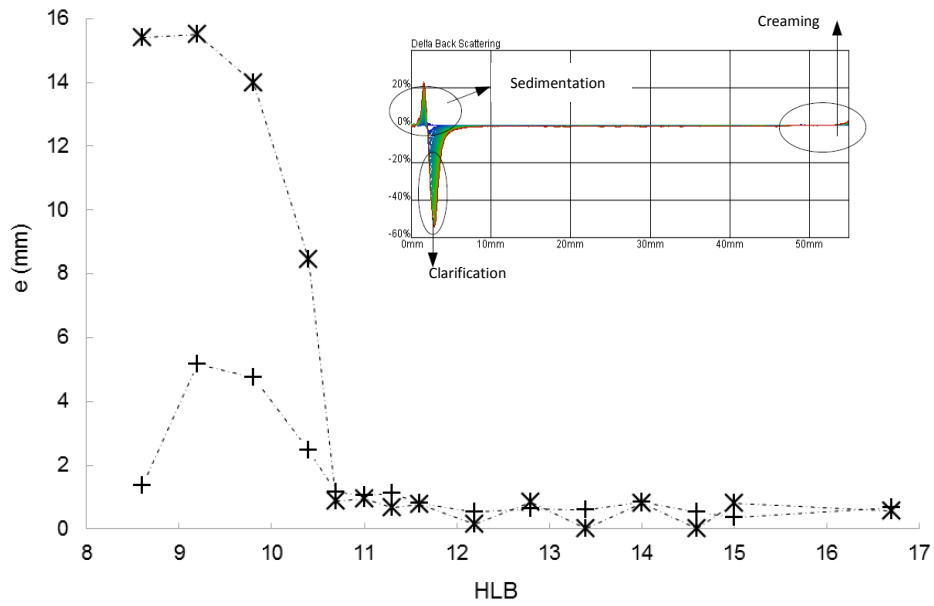
(preparation), □ - 21 days and Δ - 50 days.

Figure 2. Thickness of the layer of sedimentation (+) and creaming (✳) phenomena in the emulsion 60 minutes after preparation for different HLB values of the surfactant. Inner box: Backscattering profiles of the emulsion prepared with a HLB mixture value of 10.7, taking as reference the backscattering scan at time 0. Source data for the determination of the thickness of the referred layers.
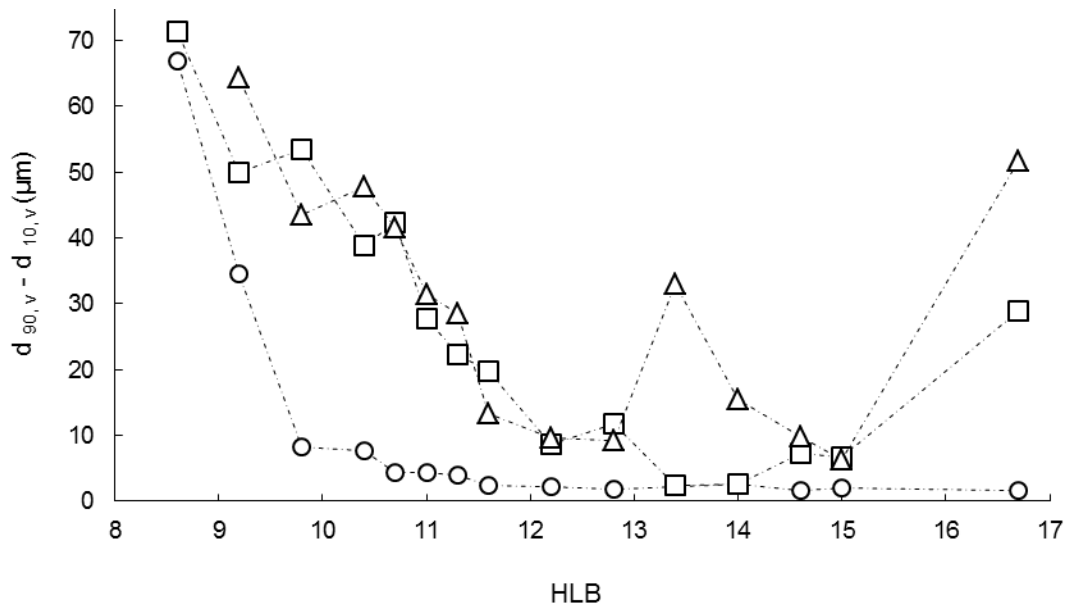
Figure 3. Width of particle size distribution ($d_{90,v}$- $d_{10,v}$) for different HLB values. Evolution with time: ○ - 0 days (preparation), □ - 21 days and Δ - 50 days.
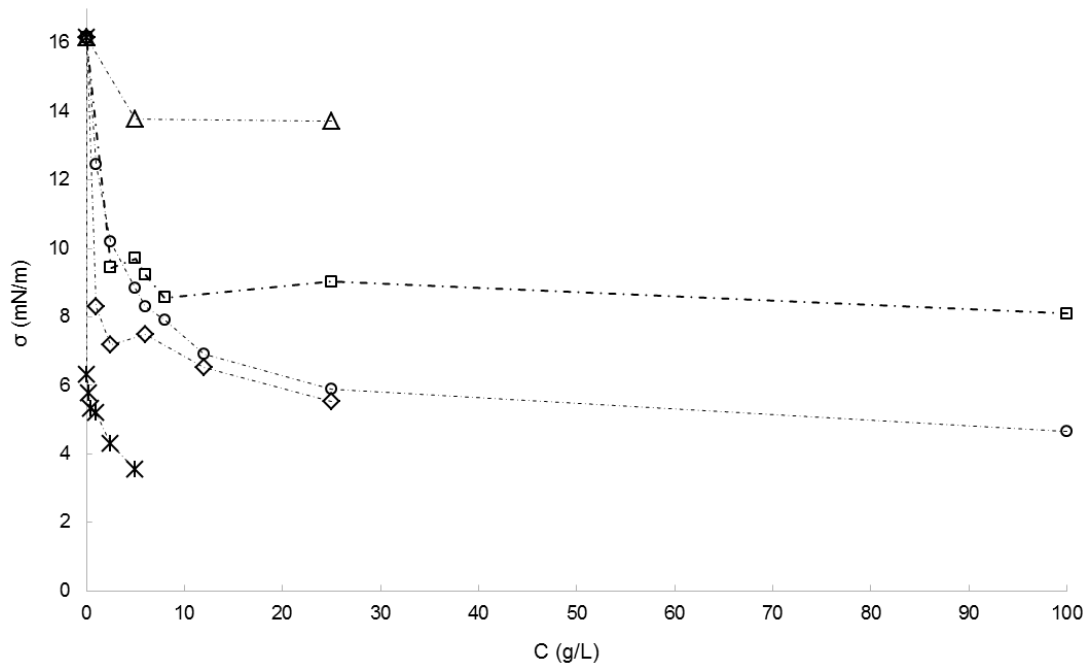
Figure 4. Reduction in interfacial tension water/ rosemary EO with the addition of surfactants:

OSA-1 (□), OSA-2 (◇), OSA-3 (Δ), OSA-4 (○) and Tween 80 (＊).