1 **Cereal bran protects vitamin A from degradation during**
2 **simmering and storage**

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11 **Abstract**

12 Food supplementation with vitamin A is an efficient strategy to combat vitamin A deficiency.
13 The stability of vitamin A during cooking and storage is, however, low. We here show that
14 cereal bran protects retinyl palmitate (RP) during simmering and storage. Native wheat bran
15 stabilized RP the most during simmering. About 75% RP was recovered after 120 min of
16 cooking, while all RP was lost after 80 min in the absence of bran. Heat-treated rice bran
17 protected RP the best during forced storage, with a 35% recovery after 8 weeks. RP was
18 degraded entirely in the absence of bran in less than one week. Results suggested that the
19 physical entrapment of oil within the large wheat bran particles protects RP from the action
20 of water and pro-oxidants during simmering. During storage, the high amount and diversity
21 of lipid components present in rice bran are presumably responsible for its protective effect.

22

23 **Keywords:** Vitamin A, Retinyl Palmitate, Food Processing, Cereal Bran, Stabilization, Protection

24 1. Introduction

25 Vitamin A deficiency (VAD) is **still a significant health issue**, especially in Africa and South-
26 East Asia, affecting mainly preschool children and pregnant women. It is estimated that 250
27 million preschool children are vitamin A deficient (WHO, 2018). A major consequence of
28 VAD in children is preventable blindness, with an estimated 250,000 to 500,000 vitamin A-
29 deficient children becoming blind every year (WHO, 2018). VAD also causes growth
30 impairment and modification of epithelial cell functionality, and increases the risk of disease
31 and **premature death** from severe infections (Lima, Soares, Lima, Mota, Maciel, Kvalsund,
32 **et al., 2010; Sommer, 2001; West, 2002; Zhiyi, Yu, Guangying, David, & Song Guo, 2018**).
33 Additionally, VAD in women of reproductive age **increases** morbidity and mortality during
34 pregnancy and the early postpartum period (Christian, West, Khatry, Katz, LeClerq,
35 Kimbrough-Pradhan, et al., 2000; West, 2002).

36 Vitamin A belongs to the group of fat-soluble vitamins. *In vivo*, it is found as free alcohol
37 (retinol) or esterified with a fatty acid (retinoids) (Bates, 1995). Two important active forms
38 of retinol are retinal, the active element of visual pigment, and retinoic acid, an intracellular
39 messenger that modulates cell differentiation (Bates, 1995). Liver and fish oil are the richest
40 animal sources of vitamin A. While plant-based foods do not contain vitamin A, many fruits
41 and vegetables contain carotenoids such as β -carotene and β -cryptoxanthin. These function
42 as provitamin A because they are converted into retinol during absorption (Bates, 1995).
43 Unfortunately, staple foods such as white rice and refined wheat products lack provitamin A
44 (Ortiz-Monasterio, Palacios-Rojas, Meng, Pixley, Trethowan, & Peña, 2007; Paine, Shipton,
45 Chaggar, Howells, Kennedy, Vernon, et al., 2005). Therefore, VAD in Asia is associated
46 with the poverty-related predominant consumption of white rice (Paine, et al., 2005). This
47 underpins the importance of human food supplementation with vitamin A to prevent VAD.
48 Fortification of suitable foods with vitamin A is a well-recognized strategy to solve VAD in
49 many parts of the world, specifically in developing countries (Fávaro, Ferreira, Desai, &

50 Dutra de Oliveira, 1991; Lee, Hamer, & Eitenmiller, 2000). Several studies, conducted in
51 Africa and Asia, showed that supplementation of vitamin A in the diet reduces infant
52 mortality in ranges from 34 to 54% (Rahmathullah, Underwood, Thulasiraj, Milton,
53 Ramaswamy, Rahmathullah, et al., 1990; Ross, Dollimore, Smith, Kirkwood, Arthur, Morris,
54 et al., 1993). Food products such as sugar (sucrose) (Arroyave, Mejía, & Aguilar, 1981), soy-
55 bean oil (Fávaro, Ferreira, Desai, & Dutra de Oliveira, 1991) and rice (Murphy, Smith,
56 Hauck, & O'Connor, 1992) have been explored as potential ingredients for vitamin A
57 fortification. Rice and wheat-based foods are ideal candidates for vitamin A fortification
58 because they are staple foods.

59 A common source of vitamin A for food and pharmaceutical supplementation is retinyl
60 palmitate (RP). However, RP is quite sensitive to environmental conditions such as
61 temperature, light and oxygen exposure (Tolleson, Cherng, Xia, Boudreau, Yin, Wamer, et
62 al., 2005), with the latter playing a crucial role. This means that the stability of RP during
63 food processing and storage, when it is exposed to temperature, light and oxygen, is rather
64 low. Loss of RP reduces the quantity of RP available for uptake by consumers.

65 A lot of the previous research on vitamin A stability focused on the degradation of
66 carotenoids instead of RP. Since RP and β -carotene have similar chemical structures and
67 properties (Loveday & Singh, 2008), the general theory for the degradation of β -carotene
68 may also hold for RP degradation. Carotenoids are highly reactive towards molecular oxygen
69 and are therefore quite rapidly degraded in food products during storage, even at reduced
70 temperatures (Kim, Strand, Dickmann, & Warthesen, 2000; Mordi, Walton, Burton, Hughes,
71 Ingold, & Lindsay, 1991). Singlet oxygen, atmospheric oxygen, peroxy and (free) radicals
72 are described as the potential mediators for carotenoid and retinoid degradation (Kim, Strand,
73 Dickmann, & Warthesen, 2000; Mordi, Walton, Burton, Hughes, Ingold, & Lindsay, 1991;
74 Yamauchi, Miyake, Inoue, & Kato, 1993). The sensitivity of RP to light is due to

75 photochemical reaction of retinoids proceeding through different routes, such as
76 photoisomerization, photopolymerization, photooxidation, and photodegradation
77 (Mousseron-Canet, 1971; Tolleson, et al., 2005). The photodegradation of RP and the type
78 of decomposition products that are formed can be influenced by a number of factors including
79 the concentration of RP, dosage and wavelength of the light, irradiation time and the presence
80 of other chemicals (Allwood & Martin, 2000; Tolleson, et al., 2005). When it comes to food
81 products, cooking, frying and storage represent major issues for RP stability (Fávaro,
82 Ferreira, Desai, & Dutra de Oliveira, 1991; Lee, Hamer, & Eitenmiller, 2000). Previous
83 research showed that part of the RP present in fortified rice was lost during cooking. The
84 extent of RP loss depended on the cooking method that was used (Lee, Hamer, & Eitenmiller,
85 2000). In the same study, the stability of RP in rice, stored at two different temperatures and
86 relative humidities, was more affected by temperature than by humidity (Lee, Hamer, &
87 Eitenmiller, 2000). Experiments with RP fortified soybean oil for frying potatoes indicated
88 that there was a progressive loss of RP in oil and that this loss was dependent on the frequency
89 of reuse of the frying oil (Fávaro, Ferreira, Desai, & Dutra de Oliveira, 1991).

90 Overall, in aqueous solutions, the solubility of retinoids is low because of their low polarity,
91 but their degradation is rapid (Semenova, Cooper, Wilson, & Converse, 2002). The solubility
92 and stability of retinoids can be improved by incorporating them into colloidal carrier
93 particles (Bates, 1995; Loveday & Singh, 2008). The carriers currently available are single
94 and double emulsions, liposomes, solid lipid nanoparticles and polymeric micro- or
95 nanoparticles. Additionally, complexing retinoids with molecular carriers, such as
96 cyclodextrins and specific proteins, can improve their stability (Loveday & Singh, 2008).
97 However, these techniques are laborious, costly and difficult to apply at industrial scale for
98 food fortification. Recently, a study by Moccand and others (2016) reported that dilution of
99 RP in triglycerides is a natural and appropriate way to stabilize it (Moccand, Martin, Martiel,

100 Gancel, Michel, Fries, et al., 2016). This study also indicated that unsaturated fats generate
101 more oxidation products such as radicals and peroxides, leading to quicker degradation of
102 RP.

103 As the degradation of RP during processing and storage of food products results in a loss of
104 nutritional value, there is a need for alternative strategies to stabilize vitamin A when
105 incorporated in food products. In this study, we explored the potential of cereal bran for RP
106 stabilization. Despite its low price and nutritional value (e.g., fibres, antioxidants), no
107 attention has been paid to cereal bran as a possible stabilizing carrier for RP in food products.
108 Therefore, we studied the effect of rice, oat and wheat bran addition on RP degradation during
109 cooking. We characterized the bran samples in terms of moisture content, particle size, oil
110 binding capacity and lipid content and composition, to identify possible correlations between
111 specific bran properties and RP stability. The effect of cereal bran on RP stability during an
112 accelerated shelf life experiment was analyzed as proof of concept.

113

114 **2. Materials and Methods**

115 *2.1 Materials*

116 Heat stabilized non-defatted rice bran was obtained from Herba Ingredients (Seville, Spain).
117 Dossche Mills (Deinze, Belgium) supplied the untreated wheat bran. Oat bran was procured
118 from Grain Millers (Eugene, Oregon, USA). Retinyl palmitate [(RP), 1 800 000 USP units/g],
119 was obtained from Sigma-Aldrich (Bornem, Belgium). Palm oil was from SANIA (Abidjan,
120 Ivory Coast). Sunflower oil was from Vandemoortele (Izegem, Belgium). Butan-1-ol was
121 from Chem-Lab (Zedelgem, Belgium). All other reagents, solvents and chemicals were of
122 analytical grade and obtained from Sigma-Aldrich (Bornem, Belgium).

123

124 2.2 Methods

125 2.2.1 Simmering experiment

126 A mixture of RP and palm oil (ratio 1:125) was added to the different bran samples (ratio
127 1:4) and mixed in a 10-gram pin mixer for 10 min. Deionized water (250 ml) at 85 °C was
128 added to 0.28 g of the mixture and blended in a Waring blender model 7011HS (Waring
129 Commercial) for 3 min. These ratios and amounts were selected to have a realistic quantity
130 of vitamin A, corresponding to 15-30% of the RDI, in the aqueous blend and have optimal
131 bran – oil mixing. Four aliquots of 15 g of the obtained aqueous blend were weighed into
132 four glass tubes. One tube was cooled down and stored at 4 °C until further experiments. The
133 other three tubes were placed in a water bath at 98 °C for 40, 80 and 120 min. At each time
134 point, the tubes were cooled down with cold water, and 15 µl of Tween 20 was added to each
135 of them. They were vortexed for two times 5 sec and placed subsequently at 4 °C until further
136 use. A control sample containing RP and palm oil was used to analyze the stability of RP in
137 the absence of cereal bran. For this sample, 0.056 g mixture of RP and palm oil (ratio 1:125)
138 was added to deionized water (250 ml) at 85 °C and vortexed (2x5 sec) to obtain a
139 homogenous sample. This mixture was used in a simmering experiment at 98 °C for 40, 80
140 and 120 min. The RP content of the samples was analyzed as described below (2.2.3). The
141 percentage RP recovered after 40, 80 and 120 min of simmering was calculated by dividing
142 the concentration of RP measured in these samples with the concentration of RP measured
143 in the uncooked sample (0 min):

$$144 \quad \% \text{ RP recovery} = \frac{\text{RP concentration cooked sample } (\mu\text{g/g})}{\text{RP concentration uncooked sample } (\mu\text{g/g})} * 100$$

145 Experiments were performed in duplicate at two different days to take day-to-day variability
146 into account.

147 2.2.2 Shelf life study

148 For the shelf life study, the RP - oil - bran ratios were kept the same as for the cooking
149 experiments: RP was mixed with palm oil at a ratio of 1:125, and 10 g of this RP-containing
150 oil was mixed with 40 g of bran. After obtaining a homogenous mixture, the samples were
151 divided into six parts. One part was frozen at -20 °C and served as a starting point. The other
152 five portions were stored in an acclimatized room at 60 °C and 68% relative humidity for 1,
153 2, 3, 6 and 8 weeks. The RP content of the different samples was quantified as described
154 below (2.2.3). For all experiments, a control sample without bran was prepared and incubated
155 under the same conditions. The percentage of RP recovery was calculated by dividing the
156 measured RP concentration in the stored samples by the measured RP concentration at the
157 starting point:

$$158 \quad \% \text{ RP recovery} = \frac{\text{RP concentration stored sample } (\mu\text{g/g})}{\text{RP concentration starting point } (\mu\text{g/g})} * 100$$

159 Experiments were performed in duplicate.

160

161 2.2.3 Quantification of retinyl palmitate (RP)

162 RP quantification was performed as described in AOAC Official Method 2012.09 (AOAC,
163 2012) with some modifications on sample preparation as described below.

164 RP was first saponified. To this end, 12.0 g of each sample taken at different time points
165 during simmering, was mixed with sodium ascorbate (0.40 g), sodium sulphide (0.40 g),
166 potassium hydroxide (2.80 g) and absolute ethanol (20.0 mL) in a 50 mL brown Erlenmeyer.
167 The mixture was flushed with nitrogen gas and placed on a magnetic stirrer overnight for
168 cold saponification. For the storage experiment, bran samples (0.28 g) or control samples
169 (0.056 g) taken at different time points were mixed with 250 mL deionized water (85°C)
170 using a blender or vortex as described for the simmering experiment. Subsequently, 12.0 g
171 of each of these mixtures was taken, and saponification was performed as described above.

Hereafter, sodium dodecyl sulfate (0.75 g) and milli-Q water (to a volume of 50 mL) were added, and the mixture was shaken for 1 min with a vortex. An aliquot of 20 ml of this saponified mixture was pipetted over a pre-filled Chromabond XTR (Macherey-Nagel, Germany) cartridge. After 15 min, the cartridge was washed with *n*-hexane (100 ml). The run-through was collected in a 100 ml brown Erlenmeyer. The solvent was evaporated using the 2-25 CDplus Rotational Vacuum Concentrator (RVC, Christ, Osterode am Harz, Germany). The residue was dissolved in 5.0 ml of *n*-hexane and analyzed by HPLC. The HPLC system (Shimadzu, Kyoto, Japan) consisted of an LC-20AT pump, a DGU-20A3 degasser, a SIL-20ACHT autosampler, an SPD-10AVP UV-VIS detector and a CTO-20AC column oven. The system was equipped with a Waters Spherisorb Silica analytical column (5 μ m, 4.6 x 250 mm). The mobile phase was a solution of 1% (v/v) 2-propanol in *n*-hexane, degassed in an ultrasound bath. The injection volume was 20 μ L. The calibration curve was made with an *all*-trans-retinol standard solution and showed linearity in the range of 37 ng/ml to 5.9 μ g/ml. The LOQ and LOD were estimated at 25 μ g RP/g sample and 3 μ g RP/g sample, respectively.

187

188 2.2.4 Determination of moisture content

189 The moisture content of the bran samples was measured according to AACC International
190 Method 44-15.02 (AACC, 2000), based on air-oven drying.

191

192 2.2.5 Determination of particle size

193 Bran particle size distribution within a range from 0.04 μ m to 2000 μ m was analyzed with a
194 laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, Miami, USA) using the
195 dry module. One-fourth of the sample holder was filled with the sample, and analysis was
196 performed according to the guidelines of the device. The Fraunhofer diffraction method was

used for particle size distribution analysis, and the volume-weighted diameters were calculated with the Beckman Coulter software. Due to the limitation of the equipment with regard to the measurement of large particle sizes, the particle size distribution of wheat bran was measured using sieves according to the method described by Jacobs and co-workers (Jacobs, Hemdane, Dornez, Delcour, & Courtin, 2015). Experiments were performed in triplicate.

203

204 2.2.6 Determination of the oil-binding capacity of bran

The methods for the determination of strong and total oil-binding capacity were adapted from procedures for the determination of strong and total water-binding capacity as described by Roye and co-workers (Roye, Bulckaen, De Bondt, Liberloo, Van De Walle, Dewettinck, et al., 2020) and Jacobs and co-workers (Jacobs, Hemdane, Dornez, Delcour, & Courtin, 2015) by replacing water with sunflower oil.

210 *Strong oil-binding capacity (using drainage)*

Bran (50.0 mg) was weighed in QIAprep Spin Miniprep Columns (Qiagen, Hilden, Germany). Sunflower oil (700 µl) was added, and the sample was stored at room temperature for 1 hour. After the incubation period, the sample was centrifuged at 4000g for 10 min at 25 °C. The amount of oil strongly bound in/to the bran structure that could not be separated from it using centrifugal force was then determined by subtracting the initial weight of the bran sample from the weight of the sample with the amount of oil absorbed by it. Experiments were performed in triplicate.

218 *Total oil-binding capacity (without using drainage)*

Sunflower oil (10 mL) was added to 1.0 g of bran in a 50-ml falcon tube. The sample was incubated for 1 hour at room temperature and then centrifuged at 4000g for 10 min at 25 °C

(Sigma, Osterode am Harz, Germany). The oil was decanted, and the tubes were placed at a 45° angle for 15 min to remove the remaining oil from the pellet. The falcon tube was weighed, and the increase in weight of the sample gives the amount of oil that is bound by the bran. Experiments were performed in triplicate.

225

226 2.2.7 Quantification of free, bound and total lipids

227 For quantification of free, bound and total lipids, lipid extraction was performed using the
228 Accelerated Solvent Extractor (ASE) (Dionex, Thermo Scientific, Amsterdam, The
229 Netherlands). The bran samples (1.0 g) were mixed with 28 g of acid-washed sand (50-70
230 mesh particle size) and poured into a 22 mL ASE extraction cell with two cellulose filters at
231 the bottom. Hexane and water-saturated-butanol were used to extract free and bound lipids,
232 respectively. Extraction was performed at 6.9 MPa and 40 °C. Because starch does not
233 gelatinize under such conditions, no starch lipids were extracted (Chung, Ohm, Ram, Park,
234 & Howitt, 2009). A cycle consisting of heating (5 min) and static extraction (10 min) was
235 repeated three times before the ASE cell was flushed with solvent and purged with nitrogen.
236 The solvents were then evaporated with the RVC (Christ). After solvent evaporation, the
237 weight in the tubes represented the amount of free, bound and total lipids. This experiment
238 was performed in triplicate. To separate and identify the lipid compounds present in the
239 extracted lipid fractions, an optimized HPLC method using evaporative light scattering
240 detection that allows single-run separation and detection of non-polar and polar lipids was
241 used (Gerits, Pareyt, & Delcour, 2013). During sample preparation, cholesterol was added as
242 an internal standard to ensure method reproducibility. A modular HPLC system (Shimadzu,
243 Kyoto, Japan) consisting of an SCL-10Avp controller, an LC-10ADvp pump, a CTO-
244 10APvp column oven set at 40 °C, and a SIL-10ADvp auto-injector was used for the analysis
245 of lipids. A monolithic Chromolith Performance-Si column (100 × 4.6 mm inner diameter),

a quaternary gradient of the mobile phases and a running time of 35 min were used for the separation of lipids as previously described (Melis, Pauly, Gerits, Pareyt, & Delcour, 2017). Lipids were detected with an evaporative light scattering detector (ELSD 3300, Alltech, Deerfield, IL, USA) with a detector drift tube temperature of 40 °C, and a gas flow of 1.5 L/min (Gerits, Pareyt, & Delcour, 2014). A standard mixture consisting of triacylglycerols, diacylglycerols and free fatty acids (FFA) was injected in the HPLC to confirm the retention time of the components and to identify any possible shift in retention times. Data acquisition was performed with Shimadzu LC Solution version 1.23 SP1. Lipid concentrations are expressed as the areas under the curve relative to that of the internal standard.

3. Results

3.1 Stability of RP during simmering

In the first instance, the ability of rice, oat and wheat bran to stabilize RP during simmering was examined. RP in palm oil was mixed with rice, oat and wheat bran and cooked for 2 h at 98 °C. Samples were taken at different cooking times, and RP concentrations were quantified by HPLC. Results are shown in Figure 1. After 40 min of cooking, rice, oat and wheat bran stabilized RP to the same extent ($\pm$ 80% recovery of RP). However, after 120 min of cooking, the stabilizing effect of wheat bran on RP was higher (75% recovery) compared to rice and oat bran (50% recovery). In the absence of cereal bran (control sample), RP was entirely degraded after 80 min of cooking. These results show the potential of bran for RP stabilization during cooking.

3.2 Characterization of bran samples

3.2.1 Moisture content and particle size measurement

Table 1 shows the moisture content and the average particle size of rice, oat and wheat bran determined as described in sections 2.2.4 and 2.2.5. Oat bran had a larger particle size than rice bran, but wheat bran had the largest particle size (Table 1).

3.2.2 Oil-binding capacity

Two types of oil retention capacity tests were performed to evaluate the oil-binding capacity of rice, oat and wheat bran. Figure 2a shows the total amount of lipids that the bran samples can accommodate. Wheat bran showed the highest total oil-binding capacity, which was about four times higher than that of rice and oat bran. Figure 2b shows the amount of strongly bound oil that was not removed from the bran structure after centrifugation. Rice bran showed a lower amount of strongly bound oil compared with oat and wheat bran, that had a similar amount of strongly bound lipids.

3.2.3 Analysis of lipid composition

The lipid composition of rice, oat and wheat bran was analyzed to determine the lipid concentration in the bran samples and to identify the nature of these lipids. Figure 3a shows the free, bound and total lipid content [% per dry matter (dm)] of rice, oat and wheat bran. Rice bran had a lipid content of 21% dm, while oat bran samples contained around 7% dm. Wheat bran had a lipid content of 3% dm. These differences in lipid content might have an impact on the oil retention capacity of the bran and consequently, can affect the absorption of oil during mixing of bran with palm oil and RP. Next, the composition of the different lipid fractions was determined to identify potential active components, which might contribute to the stabilization of RP.

Figure 3b depicts the amount of triacylglycerols and FFA in the free lipid fraction (i.e. extracted with hexane) of rice, oat and wheat bran. Rice bran had a high free lipid content, which is mainly composed of triacylglycerols and a small fraction of FFA (0.5%). Oat bran mainly contained triacylglycerols and only a minimal amount of FFA (about 0.1 to 0.3%). Wheat bran contained a relatively high amount of FFA (0.8%) despite its low amount of free lipids (1.4%) compared to rice and oat bran.

Figure 3c shows the amount of FFA, steryl glycosides, phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI) and phosphatidylcholine (PC) in the bran samples extracted with water-saturated butanol. Some components present in the bran samples were not identified and are presented as “unknown”. A wide range of sterols and phospholipids were identified in rice, oat and wheat bran. Wheat bran contained higher amounts of steryl glycosides, a class of plant-derived sterols, compared to rice bran. Oat bran contained no or little amounts of steryl glycosides. Rice bran contained PE, PA and PI. None of these components could be identified in oat or wheat bran. Phosphatidylcholine was identified in all bran samples, with the highest amount in rice bran. Among the analyzed samples, rice bran showed the largest diversity of components, while oat bran showed the lowest number of quantified components.

3.3 Stability of RP during an accelerated shelf life experiment

The stability of RP during storage is an essential factor regarding potential applications in a final product. As proof of concept of the potential of cereal bran to stabilize RP during storage, the stability of RP mixed with cereal bran during a storage period of 8 weeks was analyzed. We chose to leave out oat bran for the shelf life study because rice and oat bran did not have a significantly different effect on RP recovery during simmering.

317 As shown in Figure 4, all RP in the control sample was degraded in less than one week, while
318 in the presence of rice bran, about 35% of RP could still be recovered after 8 weeks of storage.
319 Wheat bran demonstrated a poor stabilizing effect under the conditions used for storage,
320 which is the opposite of the observations during simmering. After one week of storage, the
321 vitamin A recovery was about 60%, and after 3 weeks, all vitamin A in the wheat bran
322 mixture was degraded.

323

324 4. Discussion

325 This study aimed to evaluate the effect of different bran samples on the stability of RP during
326 simmering and storage. Both simmering and shelf life experiments showed that RP is
327 stabilized when brought into contact with cereal bran. An opposite effect for wheat and rice
328 bran for simmering and storage was observed. As shown in Figures 1 and 4, wheat bran had
329 a strong stabilizing effect during simmering but less so during storage, while the opposite
330 was true for rice bran. It must be recalled that simmering experiments and shelf life
331 experiments are different in two aspects. Simmering takes place at a temperature about 40°C
332 higher than that used during the accelerated shelf life experiment and occurs in excess of
333 water. As a result, during simmering, degradation takes place within minutes or hours, while
334 for the shelf life experiment, degradation occurs in a timeframe of weeks. Our analyses
335 suggest that the entrapment of RP in wheat bran protects it during simmering. However, the
336 results of the shelf life experiments indicate that the protective effect of wheat bran is more
337 limited during storage for a more extended period, which might be explained by the presence
338 of lipase activity. It can be hypothesized that lipase activity, and hence the formation of FFAs,
339 prevents wheat bran from exercising its protective effect on RP during storage for a longer
340 period. Additionally, the abundance of lipid components present in rice bran seemed to have

341 a protective effect on RP during accelerated shelf-life experiments, but less during
342 simmering.

343 Characterization of the different types of bran was performed to gain more insight into the
344 intrinsic physicochemical properties of the bran samples and to identify the most important
345 ones for RP stabilization. In the sections below, hypotheses on the physical and chemical
346 mechanisms by which cereal bran can protect RP during simmering and storage are
347 discussed.

348

349 *4.1 Physical mechanisms responsible for the protection of retinyl palmitate by bran*

350 The physical characteristics that were studied are particle size and oil-binding capacity.

351 Particle size is an important functional factor which determines the specific surface area and
352 the surface properties of bran (Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin,
353 2016). These properties might have an impact on the incorporation of RP in the bran
354 structure. Additionally, it has been hypothesized that reducing the particle size of bran might
355 lead to the liberation of active components due to cell breakage (Noort, van Haaster, Hemery,
356 Schols, & Hamer, 2010). The exposure to or release of active components such as
357 antioxidants can lead to higher stability of RP. In this study, the opposite effect was observed
358 for simmering experiments. Wheat bran had a larger particle size and a higher stabilizing
359 effect on RP during simmering compared with rice and oat bran. It can, therefore, be
360 hypothesized that the larger particle size of wheat bran might lead to better incorporation of
361 oil and RP in the structure, thereby protecting it from degradation during cooking.

362 In the next step, the oil-binding capacity of rice, oat and wheat bran samples was studied.

363 Analysis of the oil-binding capacity can lead to more insight into the tendency of bran to
364 absorb oil mixed with RP and keep it bound in or to the matrix, thereby protecting RP from
365 degradation. Wheat and oat bran showed a similar strong oil-binding capacity that was higher

than that of rice bran. This might be explained by the high lipid content of rice bran (21% dm) (Figure 3). The total oil-binding capacity was the highest for wheat bran. This is probably related to its large average particle size and, therefore, more irregular stacking and entrapment of oil in between the bran particles. Indeed, it can be assumed that the amount of oil strongly bound by bran is related to the physical structure of bran, including capillary attraction (Wang, Sun, Zhou, & Chen, 2012), while the total oil-binding capacity of bran is also determined by the stacking behaviour of bran particles. Correlation tests (Figure 5) revealed that there is a significant correlation between RP stability and total oil-binding capacity (correlation coefficient=0.91, $p=0.0018$), but not between RP stability and strong oil-binding capacity (correlation coefficient=0.57, $p=0.14$). Therefore, it can be hypothesized that larger particle sizes of wheat bran lead to more direct or indirect interaction with the oil and the RP in it and that this physical interaction with or enclosure of oil in the bran protects RP from the action of water and pro-oxidants. It is known that the presence of bran strongly slows down the diffusion of water during soaking, indicating the good diffusion barrier properties of bran (Bello, Tolaba, & Suarez, 2004).

In the simmering experiments, this diffusion barrier effect is likely to be higher with wheat bran, which has a larger average particle size and binds oil better in comparison with oat or rice bran. This might explain why wheat bran is very effective in protecting RP during simmering but less during storage, where no excess water is present and chemical components might be more determining for the protection of RP.

4.2 Chemical mechanisms responsible for the protection of retinyl palmitate by bran

The chemical parameters investigated were the total amount and composition of lipids present in the bran samples. Due to the lipophilic nature of RP, chemical interactions between RP and the lipids present in bran may be responsible for the stabilizing effect. The lipid content of rice bran was the highest (21% dm), of oat bran intermediate (7% dm) and of wheat

bran the lowest (3% dm), consistent with the literature (Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008; Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin, 2016; Kaur, Sharma, Nagi, & Dar, 2012). As for lipid composition, a wide range of sterols and phospholipids were identified and quantified in the different bran samples. Rice bran contained both the largest amount and highest diversity of lipid components in comparison to wheat and oat bran. This high lipid content seemed to have an effect on RP stability during storage but less during simmering. Rice bran contained, for example, higher amounts of phosphatidylcholine compared to wheat and oat bran. It was previously shown that vitamin A binds to the lipid bilayer of phosphatidylcholine liposomes and that this binding increases its stability (Singh & Das, 1998). This can explain the higher stabilizing effect of rice bran on RP during the shelf life experiment. In addition, a higher FFA content was found in wheat bran, which is probably related to residual lipase activity present in wheat bran. Indeed, in contrast to the rice bran, the wheat bran was not stabilized by heat treatment prior to commercialization, which might explain the presence of residual lipase activity generating free fatty acids, which are known pro-oxidants (Miyashita & Takagi, 1986). Overall, the chemical composition of the bran samples, i.e. the high amount and diversity of lipid components present in rice bran and the presence of lipase activity in wheat bran leading to a higher amount of FFAs, might contribute to the opposite effect that is observed for wheat and rice bran during simmering and storage.

5. Conclusions

In conclusion, cereal bran can protect RP from degradation, but the efficiency of this protective effect depends on the cereal bran source. Wheat bran showed a higher protective effect on RP during cooking, while rice bran protected RP more efficiently during long term storage. A correlation between the total oil binding capacity of bran and RP stability was

identified for the simmering experiment. Our hypothesis for this observation is that the larger average particle size of wheat bran leads to more stacking and that the physical entrapment of oil between the bran particles protects RP from the action of water and pro-oxidants, which is important during simmering in excess water, but less during storage. It is suggested that lipase activity prevents wheat bran from exercising its protective effect on RP during storage for a more extended period. The protective effect of rice bran during shelf life experiments might be related to its chemical composition, as rice bran contains a high amount and diversity of lipid components that can protect RP from degradation. It can be assumed that chemical components are instrumental in the protection of RP during storage while slowing down the diffusion of water is more important during simmering.

Although the research performed resulted in valuable hypotheses regarding the effect of cereal bran on RP stability, the stabilization mechanisms are not fully elucidated yet. More research on the impact of the physicochemical properties and the antioxidant capacity of bran on RP stability will be performed to elucidate the mechanisms further.

Abbreviations used

VAD, Vitamin A Deficiency; RP, Retinyl Palmitate; ASE, Accelerated Solvent Extractor; FFA, Free Fatty Acids

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Notes

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Table 1. Moisture content (% w/w on as is basis) and particle size characteristics of rice, oat and wheat bran (μm). The particle size distribution for rice and oat bran was determined using a laser diffraction based particle size analyzer (dry module). The particle size characteristics of wheat bran were determined using the sieve method. Means with standard deviations of triplicate measurements are mentioned in the table.

	Moisture content (% w/w as is)	Particle size (μm)		
		Mean	Median	D ₉₀
Rice bran	9.39 ± 0.06	477 ± 22	375 ± 16	999 ± 56
Oat bran	8.84 ± 0.07	1170 ± 23	1138 ± 24	1748 ± 41
Wheat bran	12.91 ± 0.04	1510 ± 38	-	-

Figure 1: The recovery (%) of retinyl palmitate (RP) during simmering (98°C, 120 min) in the presence of rice, oat and wheat bran expressed as a percentage of the RP content measured before simmering (0 min). The control sample did not contain bran. Error bars represent standard deviations of duplicate measurements.

Figure 2: a) The total oil-binding capacity of wheat, oat and rice bran [g oil that is strongly and loosely bound by 1 g of bran] and b) the strong oil-binding capacity of wheat, oat and rice bran [mg of oil strongly bound by 100 mg of bran]. Error bars represent standard deviations of triplicate measurements.

Figure 3: a) Free, bound and total lipids content (% on bran dry matter) of rice bran, oat bran and wheat bran. Error bars represent standard deviations of triplicate measurements. b) Estimation of the concentration (% on bran dry matter) of triacylglycerols (TAG) and free fatty acids (FFA) in the free lipid fraction (i.e. the fraction extracted with hexane) of rice bran, oat bran and wheat bran. c) Estimation of the concentration (% on bran dry matter) of free fatty acid (FFA), steryl glycoside, phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI) and phosphatidylcholine (PC) in the total lipid fraction (i.e. the fraction extracted with water-saturated butanol) of rice bran, oat bran and wheat bran. The unknown fraction is the sum of different components that could not be quantified using this analysis.

Figure 4: The recovery (%) of retinyl palmitate (RP) after 8 weeks of storage at 60 °C and 68% relative humidity in the presence of rice and wheat bran expressed as a percentage of the RP content measured before storage. The control sample did not contain bran. Error bars represent standard deviations of duplicate measurements.

Figure 5: a) Scatterplot showing the correlation between the strong oil-binding capacity of bran and RP recovery after 2 h of simmering. Correlation coefficient = 0.57 ($p=0.14$). b) Scatterplot showing the correlation between the total oil-binding capacity of bran and RP recovery after 2 h of simmering. Correlation coefficient = 0.91 ($p=0.0018$). A 95% density ellipse, which graphically shows the correlation, is displayed on the scatterplot.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Supplementary Table 1. The proximate composition of wheat, rice and oat bran as supplied by suppliers. It should be noted that these values are average compositions of the brans provided by the different suppliers and do not represent the exact compositions of the batches used in this study. Moreover, analysis methods used by the different suppliers might differ. Therefore, proximate compositions are rather indicative and should not be considered as exact values for the brans used in this study.

Component	Wheat bran	Rice bran	Oat bran
Carbohydrates (%)	12%	32%	40%
Fibres (%)	50%	26%	39%
Proteins (%)	17%	15%	11%
Lipids (%)	5%	16%	4%
Ash (%)	7%	5%	4%
Moisture (%)	9%	6%	2%

Highlights

- Cereal bran **protected** retinyl palmitate from degradation during cooking and storage
- Wheat, rice and oat bran **had** a different protective effect on retinyl palmitate
- Wheat bran **had** the highest stabilizing effect on retinyl palmitate during cooking
- Rice bran **was** the best protectant of retinyl palmitate during storage