

## Tryptophan Released From Mother's Milk Has Antioxidant Properties

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**ABSTRACT:** Bioactive factors in human milk (HM) are crucial to the health of newborns, especially preterm infants. These compounds assist in reducing the oxidative stress that may occur as a result of combined exposure to supplemental oxygen and immature physiologic defenses. To identify the components in HM that contribute to its greater resistance to oxidative stress compared with infant formulae, enzymatic hydrolysates of HM were prepared, ultrafiltered, separated, and analyzed for antioxidant potential. The antioxidant activity [ $\mu\text{M}$  Trolox equivalent (TE/g)] of nondigested milk, whole digested milk, and derived ultrafiltrates were  $80.4 \pm 13.3$ ,  $159.0 \pm 5.6$ , and  $127.4 \pm 3.1$ , respectively. An HPLC fraction denoted as fraction 23 ( $5274 \pm 630 \mu\text{M TE/g}$ ) was obtained and its constituents identified as tryptophan (Trp), peptides HNPI, and PLAPQA. Scavenging activity was not observed for PLAPQA, whereas moderate activity was associated with HNPI ( $144 \pm 10.7 \mu\text{M TE/g}$ ) and very high activity to Trp ( $7986 \pm 468 \mu\text{M TE/g}$ ). Trp addition to HM and two infant formulas significantly increased formulae antioxidant properties. Trp appeared to be a powerful free radical scavenger naturally present in HM. Its antioxidant effects and potential application in the diets of infants, particularly preterm, must be examined further. (*Pediatr Res* 66: 614–618, 2009)

Oxidative stress is implicated as an underlying factor in many pathologic processes of human health disorders (1). Birth is a physiologic process associated with enhanced stress due to the transition of the fetus from a relatively hypoxic condition in the womb (2) to a relatively hyperoxic environment during early neonatal life (3). Premature infants, in particular, are often exposed to supplemental oxygen concentrations as high as 95% (4,5); the result of which is the overproduction of reactive oxygen species (ROS). Numerous health conditions among preterm infants that are linked to oxidative stress include bronchopulmonary dysplasia, retinopathy of prematurity, intraventricular hemorrhage, necrotizing enterocolitis, and renal failure; all of which can lead to subsequent long-term complications in the physical and neurologic development of the child (5,6). The term “oxygen radical dis-

ease” was proposed to represent all the conditions affecting preterm infants that may contribute to neonatal morbidity (7).

ROS can react with polyunsaturated fatty acids of cell membranes, proteins, polysaccharides, and DNA resulting in functional alterations within the cell and subsequent tissue/cellular injury (8). Several mechanisms lead to ROS overproduction in the perinatal period, including ischemia-reperfusion, increased levels of free iron, and products of the arachidonic acid cascade (9). ROS generation *in vivo* is controlled by the action of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and the presence of nonenzymatic antioxidants, such as vitamins C and E and other bioactive agents (2,10).

Human milk (HM) is recognized as the primary and optimal food for newborns. The importance of nonenzymatic, bioactive antioxidants in HM is important because endogenous infant enzyme maturation does not occur before 28-wk of gestation and the transfer of vitamin E from the mother to the fetus occurs primarily during the last trimester of pregnancy (11,12). Thus, there are limitations to the development of the antioxidant defense system in both premature and full-term newborns (9,10), which leaves them susceptible to the adverse effects of oxidative stress (2). We postulate that HM with bioactive agents is crucial to newborns, especially preterm babies, and will assist in reducing oxidative stress while physiologic defenses are underdeveloped. For example, HM itself contains antioxidant enzymes SOD, CAT, and GPx, which eliminate superoxide anion, hydrogen peroxide, and hydroperoxide, respectively, whereas vitamins C and E provide direct free radical-scavenging benefits; as well lactoferrin binds iron and prevents the generation of ROS through the Fenton reaction (2,13). Although HM has been found to have higher antioxidant content than infant formula, the complete list of active antioxidant compounds in HM is presently unknown (14,15). The identification of these naturally occurring bioactives in HM would further enhance our understand-

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**Abbreviations:** AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; CAT, catalase; HM, human milk; ORAC, oxygen radical absorbance capacity; ROS, reactive oxygen species; SOD, superoxide dismutase; TE, Trolox equivalent; TFA, trifluoroacetic acid; Trp, tryptophan

ing of the biochemistry that explains the antioxidant capabilities of mother's milk.

In this study, we modified an *in vitro* digestion procedure to process mother's milk and recovered crude digests that were further separated into more distinct fractions by column chromatography, and subsequently characterized for antioxidant activity using the oxygen radical absorbance capacity (ORAC) assay. Efforts were made to isolate and characterize the individual components of the HM digest fractions that exhibited antioxidant activity toward the peroxy radical generated by thermal lysis of 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH).

## METHODS

**Milk samples.** HM samples were obtained from three volunteer mothers who were breastfeeding their infants. The milk was collected using a mechanical breast pump (Ameda-Egnel Lincolnshire, IL) by each lactating mother during the first 4 mo and stored at  $-20^{\circ}\text{C}$ . All samples were transported to our laboratory on dry ice. Once in our laboratory, samples from each mother were thawed at room temperature, combined, freeze-dried, and then stored at  $-80^{\circ}\text{C}$  until ready for analysis.

**Chemicals and reagents.** Pepsin P-7000, pancreatin P1750, bile salts B333, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), trifluoroacetic acid (TFA), and L-tryptophan (Trp) were purchased from Sigma Chemical Co. Aldrich Canada Ltd (Oakville, Ontario). Methanol and acetonitrile HPLC grade and fluorescein were purchased from Fisher Scientific Canada (Ottawa, Ontario). High-purity water was produced in the laboratory by an Alpha-Q system (Millipore, Marlborough, MA).

**Digestion of milk samples.** Freeze-dried HM was rehydrated immediately before conducting the experiments. Because total solids in HM account for approximately 13% (16), we used 130 g of freeze-dried HM powder and diluted this to 1 L using deionized water before conducting the two-step digestion experiments. Digestion of HM was performed using modified procedures of Etcheverry *et al.* (17) and Jovani *et al.* (18). In both these studies, a pH 2.0 was used to approximate the conditions present during adult gastric digestion. We used pH 4.0 and 6.0 for the two-step digestion procedure. This change in protocol was to more closely approximate digestion in the premature infant gut and based on a study by Omari and Davidson (19), whereby these pH values were recorded in preterm infants using a four-channel probe to measure the esophageal and intragastric changes in pH after feeding. The pH of the reconstituted HM was adjusted to 5.5 with 1 M HCl, 76 mL of pepsin (4 g/100 mL of 0.1 M HCl) added, and final pH adjusted to 4.0. The sample was incubated for 30 min at  $37^{\circ}\text{C}$  with gentle shaking at 100 rpm in a MaxQ 4000 incubator (Barnstead Lab-Line), after which the pH was adjusted to 6.0 and the sample was further incubated for another 30 min. After peptic digestion, 380 mL of a 0.1 M  $\text{NaHCO}_3$  solution containing pancreatin (0.8 g/400 mL) and bile salts (4.8 g/400 mL) were added. The solution was adjusted to pH 7.0 with 1 M  $\text{NaHCO}_3$  and incubation was carried out for 2 h at  $37^{\circ}\text{C}$ . After incubation, the sample solution was heated in a water bath at  $90^{\circ}\text{C}$  for 15 min to inactivate pancreatin enzymes.

**Ultrafiltration and HPLC separations.** The digested sample was transferred to a separatory funnel and stored for 5 h at  $4^{\circ}\text{C}$  to separate the lipid and aqueous phases. An Amicon stirred cell (unit model 8400) containing a 3000 Da molecular weight cutoff membrane (Millipore, Billerica, MA) was used to separate the aqueous phase into low molecular weight ( $<3000$  Da) peptide fraction (permeate). All digested samples were freeze dried and stored at  $-80^{\circ}\text{C}$ .

HPLC separation of the 3000 Da membrane permeate was performed using a 600E Multisolute Delivery System equipped with a 5 mL manual sample loop, 100  $\mu\text{L}$  pump head, 717 autosampler, 996 PDA detector, and fraction collector III (Waters Corporation; Milford, MA). The column used was Waters Symmetry 300 C18 (5  $\mu\text{m}$   $19 \times 250$  mm). Solvent A was 0.05% TFA in water and solvent B contained 0.05% TFA in acetonitrile. The HM ultrafiltered permeate (150 mg/mL) in solvent A was filtered on 0.45- $\mu\text{m}$  membrane and 5 mL were injected onto the HPLC column. A linear gradient of solvent B ranging from 0 to 70% in solvent A during 60 min at a flow rate of 6 mL/min was used to separate peptides. Fractions were collected every min (6 mL), individually freeze dried and used for the ORAC assay.

**Oxygen radical absorbance capacity of digested HM fractions.** The assay was performed according to the literature (20,21). An FLX800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) with fluores-

cence filters (excitation 485/20 nm, emission 528/20 nm) was used and run using the KC4 software. Five Trolox concentrations between 6.25 and 100  $\mu\text{M}$  were used for calibration curve. The antioxidant control was rutin trihydrate (20  $\mu\text{M}$ ). Two concentrations (312.50 and 78.10  $\mu\text{g}/\text{mL}$ ) of crude HM samples and two concentrations (26.00 and 13.00  $\mu\text{g}/\text{mL}$ ) of HPLC fractions were used for each assay run. Data were processed as previously reported (21) and the unit of measurement expressed as Trolox equivalents (TE).

**ORAC of supplemented HM and formulae.** The effect of added Trp on the endogenous antioxidant potential of HM and milk formula was tested with a mature HM sample and two infant formulae to determine any potential matrix effects. The HM was obtained from an additional volunteer mother and the formulae were Earth's Best Organic (EBO) (Heinz, Pittsburg, PA) and Similac Alimentum Advance (SAA) (Abbott Laboratories, Montreal, PQ) purchased locally in Winnipeg, Manitoba, Canada. The formulae diluted in water according to the manufactures' instructions and thawed HM were nondigested. All samples were then diluted 125 and 250 times in phosphate buffer before ORAC assays were performed. The final Trp concentration in diluted samples was adjusted to 10.2  $\mu\text{M}$ , which allowed a detectable ORAC reading within our calibration curve.

**Identification by LC-MS/MS.** Compounds in fraction 23 were identified using an ultra Performance Acquity UPLC system coupled to a Quattro micro API 2000 MS/MS mass spectrometer both from Waters Corporation (both from Waters Corporation, Milford, MA). The UPLC included Acquity autosampler, binary pumps, and Acquity PDA detector (214 and 280 nm). Chromatography was performed using an Acquity BEH C18 column (50  $\times$  2.1 mm; 1.7  $\mu\text{m}$ ; Waters Corp). The mobile phase was composed of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile. A linear gradient from 0 to 20% B, delivered at 0.2 mL/min during 50 min, was used and the eluates were automatically transferred to the MS system.

The mass spectrometer was operated with an ESCi probe. MS regular scan analysis was performed in electrospray-positive mode: capillary voltage 3.5 kV, source temperature  $100^{\circ}\text{C}$ , desolvation temperature  $200^{\circ}\text{C}$ , cone gas 50 L/h, desolvation nitrogen gas 500 L/h, and electron multiplier 650 V. Daughter and parent spectra were obtained with a collision energy of 25 eV. Data were processed through MassLynx software (version 4.1, Waters Corp). *De novo* peptides sequencing was performed on MS/MS data using PepSeq software (version 1.2 available at <http://www.kuleuven.ac.be/regia/medchem/pepseq.htm>).

**Analysis of tryptophan in infant plasma and peptide fraction 23.** A blood sample was collected in EDTA-coated tubes from a 1-mo-old breastfed infant in a concurrent study. The sample was centrifuged ( $3000 \times g$  at  $4^{\circ}\text{C}$ ) to separate plasma and red blood cells. One hundred microliter of 10% trichloroacetic acid (TCA) solution was added to 100  $\mu\text{L}$  of plasma and the mixture was centrifuged at 10,000 rpm for 10 min at room temperature. The sample was filtered on a 0.45- $\mu\text{m}$  Millipore membrane and 10  $\mu\text{L}$  was analyzed using UPLC-MS/MS as described earlier.

Determination of Trp content of fraction 23 and undigested HM was performed by Advanced Protein Technology Centre, Ontario, Canada. Briefly, samples were reconstituted in water and digested with 4 M methanesulfonic acid followed by PITC derivatization. Trp was subsequently separated in reverse-phase Acquity UPLC BEH column (2.1 mm  $\times$  10 cm) at  $48^{\circ}\text{C}$  and detected at 254 nm.

**Statistical analysis and ethics.** All data are expressed as mean  $\pm$  SD from replicates analyzed in triplicate. One-way ANOVA was used to compare ORAC values. Statistical significance was set to  $p < 0.05$ . All statistics were completed with SPSSx (Version 16, 2007, Chicago, IL). All procedures received ethical approval by the University of Manitoba Ethics Research Review Board. For milk analysis, the review board did not require informed consent as the milk was discarded and any identifying features were removed. For blood analysis, informed consent was obtained from each mother.

## RESULTS

**Radical-scavenging activity of digested human milk, recovered fractions, and tryptophan.** The antioxidant activities of HM and different fractions recovered from *in vitro* digested HM samples, using the ORAC assay, are reported in Table 1. With the exception of the lipid fraction, all isolated fractions possessed peroxy radical-scavenging activity that was higher than that observed from the undigested whole milk sample (HM). After digestion of HM with pepsin plus pancreatin-bile salts and subsequent fractionation, the ORAC value of a low molecular weight ( $<3000$  Da) fraction was  $127.4 \pm 3.1$   $\mu\text{M}$  TE/g of the

**Table 1.** ORAC values of digested pepsin and pepsin-pancreatin digested HM fractions

Sample	ORAC ( $\mu\text{M Trolox/g}$ )
HM	$80.4 \pm 13.3^*$
HM P + P (lipid)	$49.8 \pm 2.5^\dagger$
HM Pepsin	$110.8 \pm 5.4^\ddagger$
HM Pepsin >3000 Da	$200.1 \pm 3.9^\S$
HM Pepsin <3000 Da	$48.6 \pm 2.5^\dagger$
HM P + P	$159.0 \pm 5.6^  $
HM P + P >3000 Da	$212.3 \pm 11.1^\S$
HM P + P <3000 Da	$127.4 \pm 3.1^  $

ORAC: mean  $\pm$  SD.

\* Values in the column with different symbols indicate significant differences ( $p < 0.05$ ).

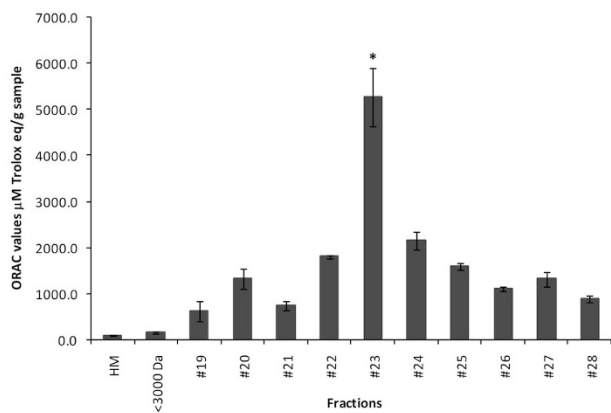
$^\dagger$  HM P + P (lipid): lipid from pepsin/pancreatin digested HM.

$^\ddagger$  HM Pepsin: pepsin digested.

$^\S$  HM Pepsin >3000 Da: pepsin digest retained on 3000 Da membrane.

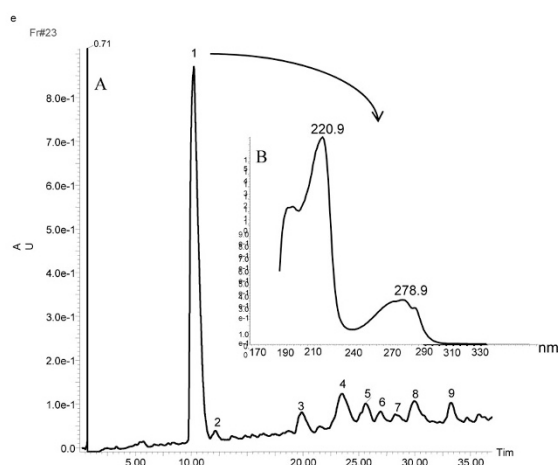
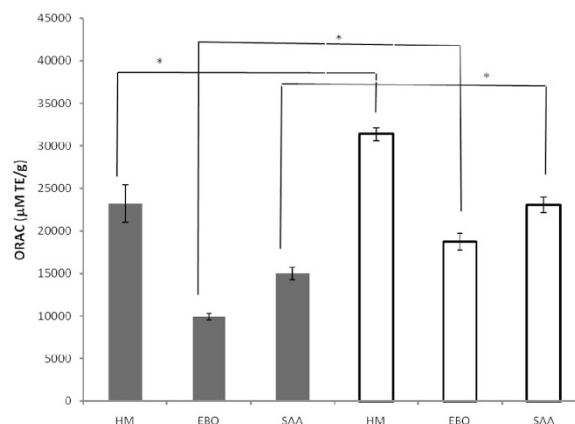
$^||$  HM Pepsin <3000: pepsin digest, permeate from 3000 Da membrane.

$^||$  HM P + P <3000 Da: pepsin/pancreatin digest, permeate from 3000 Da membrane.

**Figure 1.** ORAC values (mean  $\pm$  SD) of HM, ultrafiltered permeate (<3000) and its fractions derived from HPLC. Statistical significance  $*p < 0.01$  compared with all other fractions.

freeze-dried sample. We concentrated on this fraction because it would likely contain molecules that are potentially less susceptible to further digestion and may have higher bioavailability, as they may be capable of crossing neonatal intestinal cell barriers.

To identify the fraction of the digested HM sample with the highest possible bioactivity, the low molecular weight fraction was subjected to reverse-phase HPLC separation, as described in the Methods section. Fifty-five fractions were collected, freeze dried, and evaluated for antioxidant activity using the ORAC assay. The most potent fraction was found to be fraction 23 (Fig. 1), with an ORAC value of  $5274 \pm 630 \mu\text{M TE/g}$  of freeze-dried sample. The ORAC value of this fraction was markedly higher than both that of undigested HM ( $80.4 \pm 13.3 \mu\text{M TE/g}$ ) and the crude fraction obtained after digestion but before HPLC separation ( $127.4 \pm 3.1 \mu\text{M TE/g}$ ). Trp was identified and purchased from Sigma Chemical Co. Aldrich Canada Ltd for further testing. Two peptides identified specifically as HNPI (peak 2) and PLAPQA (peak 4) in fraction 23 were synthesized by GenWay Biotech, Inc (San Diego, CA) and tested for ORAC-scavenging activity. HNPI was

**Figure 2.** UV chromatogram (214 nm) of fraction 23 (A) and online UV spectrum (B) from Acquity UPLC system. Peaks: 1 = Trp; 2 = HNPI; 4 = PLAPQA.**Figure 3.** ORAC values of nonsupplemented (black) and supplemented milk (white) with  $10.2 \mu\text{M Trp}$ . HM: human milk, EBO, Earth's Best Organic; SAA, Similac Alimentum Advance. Statistical significance  $*p < 0.01$ .

found to have an ORAC value of  $114.0 \pm 10.7 \mu\text{M TE/g}$ , whereas no ORAC activity could be detected for PLAPQA.

**Identification of compounds in fraction 23.** Fraction 23 was analyzed by reverse-phase HPLC coupled to Quattro Micro API electrospray mass spectrometry and the UV chromatogram at 214 nm is shown in Figure 2. One major peak with a retention time (RT) of 9.47 min (here referred to as compound 1) and approximately eight other smaller peaks were identified on the chromatogram. Compound 1 was also present at 280 nm, indicating the possible presence of Trp, tyrosine (Tyr), or phenylalanine (Phe), three amino acids that contain an aromatic ring and absorb at 280 nm. The UV spectrum of compound 1 had an absorption maximum at 279 nm, a side peak at 288 nm, and a shoulder at 271 nm; which is common to that reported for Trp (22). From the Quattro MS/MS analysis, the molecular peak was observed at  $m/z$  205.3248 for compound 1, which is identical to the mass of Trp. To confirm the nature of compound 1, a standard L-Trp was analyzed concomitantly with fraction 23. RTs, UV chromatograms/spectra, and MS data were all identical. From this information, we concluded that compound 1 is free Trp.



*De novo* peptide sequencing using the PepSeQ software version 1.2 was performed to determine the sequence of other peaks in peptide fraction 23. Chromatography peaks 2 and 4 were identified as peptides HNPI and PLAQPA, respectively. They are likely derived from human  $\beta$ -casein subunits and have been reported before from digested HM samples (23). MS/MS data of peaks 5 to 9 showed that they were peptides composed of five to nine amino acids. The final amino acid sequences of these peptides have not yet been determined.

**Antioxidant activity of fraction 23 is due to tryptophan.** We evaluated the antioxidant activity of fractions 20 to 26 and found that respective ORAC values for these peptides were proportional to the content of Trp based on the area from the UV chromatograms. We also evaluated the antioxidant activity of fresh mother's milk and two infant formulas, EBO and SAA, both before and after supplementation with 10.2  $\mu$ M Trp (Fig. 3).

**Tryptophan in infant plasma.** A plasma sample from a 1-mo-old breastfed infant in a concurrent study was analyzed by UPLC-MS/MS. The presence of Trp in neonate plasma was confirmed by comparison of RT, UV chromatogram, and MS data with that of fraction 23 and standard Trp.

Among a few selected peptide fractions, fraction 23 was found to contain the highest Trp content at 7.43% of dried fraction. This level of Trp was approximately 50 times more concentrated than undigested HM, which contained 0.14% (db) Trp.

## DISCUSSION

In this study, an *in vitro* digestion on human mother's milk was performed to simulate the infant's actual digestion. We recovered free Trp from the digested milk and found that it is a potent antioxidant in crude fractions, which were purified by HPLC separation and analyzed by tandem mass spectrometry. The high concentration of Trp in fraction 23 indicates that this amino acid may be contributing to the high antioxidant activity of this fraction.

HM contains a relatively higher content of Trp than do both soy- and milk-based infant formulas (24). Trp concentration of HM protein is about 2.2–2.4% compared with 1.3% in cow milk protein and approximately 1.7% in whey protein-supplemented infant formulas (25). The difference in Trp content of HM and infant formula is mainly due to the  $\alpha$ -lactalbumin portion of whey protein. Cow milk has a whey-casein ratio of approximately 20:80, which is distinctly different from the 60:40 whey-casein ratio in HM (26). Milk-based infant formulae are produced by adding whey protein concentrate to skimmed cow's milk powder to yield a similar whey to casein ratio as that of HM. This modification results in an amino acid composition that is still quite different from that of HM protein, especially in terms of the Trp content (27). Trp is a precursor in the synthesis of the neurotransmitter serotonin, which is involved in the regulation of appetite, circadian rhythm, and affective reaction control (28). The metabolite of Trp, 5-hydroxy-Trp increases growth hormone (29) and stimulates the motility of the gastrointestinal tract. Serotonin synthesis is directly dependent on the availability of

the specific precursor (Trp) and on the nutritional status of individuals (30). Serotonin can, in turn, be used for the synthesis of the vitamin nicotinic acid (B3), which prevents pellagra (25).

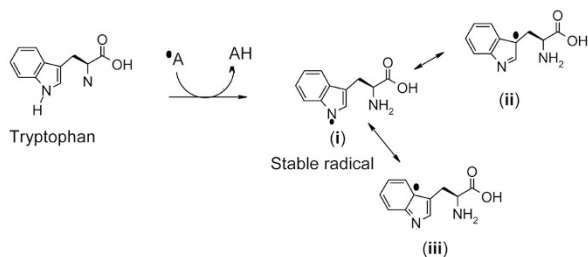
Trp is a limiting amino acid in human nutrition and to increase its concentration in the infant diet,  $\alpha$ -lactalbumin-enriched formulae may be given to term infants (31). Plasma Trp concentrations of infants fed these enriched formulae do not differ from those of breastfed infants (31). A number of other studies have supplemented free Trp to formulas. Steinberg *et al.* (32) fed healthy term infants formulae supplemented with 294, 588, and 882  $\mu$ M/L of free Trp and reported shorter sleep latency in the Trp-fortified formula groups. In a randomized intervention trial, Raiha *et al.* (33) found no difference in the growth patterns between the control group and the group receiving formula fortified with both  $\alpha$ -lactalbumin and free Trp. Fazzolari-Nesci *et al.* (34) also observed that infants fed formulae supplemented with 10 mg Trp/dL had plasma Trp concentrations similar to breastfed infant. Although formulae were supplemented with either  $\alpha$ -lactalbumin or free Trp in these former studies, the researchers did not measure the antioxidant properties of fortified formulae. Thus, direct comparison with our results is not impossible.

Only one study has evaluated the antioxidant properties of amino acids, including Trp (35). Katayama and Mine analyzed the inhibiting effects of Trp and other amino acids on H<sub>2</sub>O<sub>2</sub>-induced IL-8 secretion in CaCO<sub>2</sub> cells. Melatonin, an endogenously produced Trp metabolite was found by another group to be a scavenger of a number oxygen reactive species (hydroxyl radical, singlet oxygen, and hydrogen peroxide) and nitrogen reactive species (nitric oxide and peroxy nitrite anion), when analyzed *in vitro* and *in vivo* (36). The finding from the study of Katayama and Mine (35) complements the ORAC assay and reinforces the need to further examine Trp for its ability to reduce oxidative stress, particularly in infants.

Hernandez-Ledesma *et al.* (23) performed *in vitro* digestion of HM and formulas and identified 23 peptides, including HNPI and PLAPLQA, which we also recovered from fraction 23 in this study. Eleven of those peptides were also evaluated for angiotensin-converting enzyme inhibitor activity and antioxidant activity, using the ABTS assay (19). However, the peptide HNPI was not included in these tests, so that the scavenging activity of this peptide according to ORAC and ABTS assays cannot be compared. As in this study, no antioxidant activity was observed for PLAPQA in the ABTS assay (23).

The Trp released from digested HM, as assessed through an *in vitro* model of a preterm infant's gastrointestinal tract, was identified as a potent radical-scavenging molecule. Its structure contains a ring that can stabilize radicals through resonance or delocalization (Fig. 4), thus enabling it to break radical chain reactions. Although two others amino acids, namely tyrosine (Tyr) and phenylalanine (Phe), also possess ring structures, they were not detected in PF23 in this study. However, Tyr may still be present in the fractions with lower activities so further testing is warranted.

Little work has been performed on the interaction of Trp with other pro- or antioxidants in either the infant diet or the



**Figure 4.** Resonance forms of the Trp radical. (i) Trp is a stable radical because its unpaired electron is delocalized (*e.g.*, associated with more than two atoms) or because it has resonance forms (i–iii).

infant metabolism. Katayama and Mine (35) reported that a key antioxidant enzyme, glutathione S-transferase, was induced with Trp pretreatment in a cell culture model. Pazos *et al.* (37) reported that Trp is the amino acid with the highest antiradical activity. In a rat study, Feksa *et al.* (38) reported that Trp, vitamin C, and vitamin E interacted to modulate the oxidant effects of Trp. Clearly, more work needs to be done in this area because HM is rich with many factors that interact to modulate effects seen one molecule at a time.

In conclusion, our results indicate that the amino acid Trp may have a function beyond protein quality and the synthesis of important molecules, including serotonin, melatonin, and niacin. Trp is a powerful free radical scavenger that should be examined further for its affinity to reduce oxidative stress, especially in preterm infants whose endogenous antioxidant defense systems are immature at birth.

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