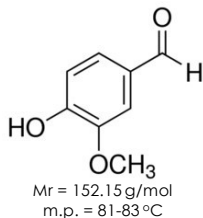


## Abstract

The vanilloids are a family of natural products containing the vanillyl group. Notable members of the group are vanillin, found in vanilla pods and capsaicin, found in chillies. Both compounds are extensively used as scents and as food flavourings. Less than 1% of vanillin used commercially is natural, while the rest is synthesized from a range of petrochemical precursors. Tests have been conducted to ascertain whether vanilla flavoured products contain natural or synthetic vanillin. The composition of extracted samples has been confirmed via melting point, GC-MS and NMR spectroscopy. Samples of other vanilloids have also been extracted from a range of natural sources and links between the biochemistry and solubility, stability and reactivity are explored. Structure activity relationships point towards the significance of the para-hydroxyl group for binding to receptive sites, offering insight into potential modes for enhancement or blocking of biological pathways.

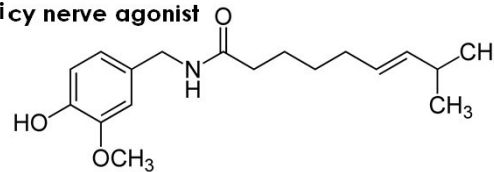
## Vanillin – the world's favourite flavour

- Native vanilla extract contains many hundreds of compounds
- Vanillin is phenolic aldehyde found in vanilla beans
- White, air stable, aromatic, crystalline solid
- Around 80% of natural vanilla grown in Madagascar
- Less than 1% of vanilla used globally is extracted from pods – the rest is synthesised
- Common synthetic starting materials include guaiacol and lignin
- In February 2015, Nestle announced intentions to go "all natural" causing a world-wide surge in the price of natural vanilla extract!



## Capsaicin – spicy nerve agonist

Mr = 305.42 g/mol  
m.p. = 62-65 °C



- Found in spicy foods such as chili peppers
- Same vanillyl core structure but with long hydrophobic aliphatic side chain
- Stimulates the TrpV1 receptor in mammals giving a feeling of heat and a piquant taste
- Commonly used in pepper sprays as well as treatments for neuropathic pain?



## Identifying solvent for vanillin extraction

Solubility of commercially sourced vanillin in green solvents investigated.  
Method:

- Solvent (100 cm<sup>3</sup>) was measured into a clean conical flask using a volumetric pipette. A stir bar was added. The mass of the flask was recorded.
- Solid, crystalline vanillin was slowly dissolved until crystals persisted.
- The flask was reweighed to determine the amount dissolved.

Solvent	Solubility (g/cm <sup>3</sup> )
Water	0.010
Ethanol	0.285
Ethyl Acetate	0.242
Butan-1-ol	0.142

- Vanillin is sparingly soluble in water but freely soluble in ethyl acetate and short-chain alcohols.
- Ethyl acetate chosen for aqueous extraction on account of immiscibility with water and ease of drying.

## Extracting vanillin from flavoured products

- Two products chosen to compare by aqueous extraction:



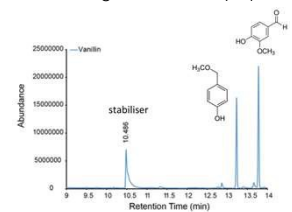
- Vanilla sugar – listing vanillin and sucrose as the only ingredients
- Madagascan Vanilla Extract – an aqueous ethanol solution of vanilla extract

Method:

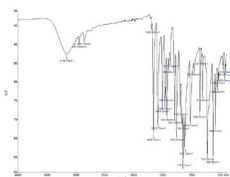
- Test sample (80g vanilla sugar or 20 cm<sup>3</sup> of vanilla extract) transferred to a conical flask and suspended in ethyl acetate (100 cm<sup>3</sup>). Stirred vigorously overnight.
- Suspension transferred to a separating funnel and extracted with deionized water (3 x 75 cm<sup>3</sup>).
- Organic layer dried with magnesium sulfate and evaporated to dryness.  
Yield: Vanilla sugar – 1.02g (1.28% by mass, white solid on standing)  
Extract – 0.82g (4.1% by mass, viscous brown oil)

## Analysis of vanillin extractions by GC-MS and FT-IR

- Samples submitted to gas chromatography-mass spectrometry
- Vanilla sugar showed only 2 peaks – vanillin and solvent stabiliser



- Spectrum for vanilla extract showed two main peaks and several smaller peaks
- 4-(Methoxymethyl)phenol present as major impurity
- Baseline peaks could not be identified



- Identify of vanillin confirmed by <sup>1</sup>H NMR and FT-IR spectroscopy

## References

- M. M. Bomgardner "The problem with vanilla" CE&N News, Volume 94, Issue 36, p. 38-42 (2016)
- H. J. McQuay "Topical capsaicin for chronic neuropathic pain in adults" Cochrane Database of Systematic Reviews 2009, Issue 4. Art. No.: CD007393

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## Extraction of capsaicin from various sources

- Samples of chopped whole chillies, chilli flakes and chilli powder soaked in ethyl acetate for 1-2 weeks.
- Sample washed with deionized water, dried with magnesium sulfate and evaporated to dryness.



Sample	Starting mass (g)	Residue mass (g)	Yield (% mass)
Red chillies (whole)	11.03	0.61	6%
Green chillies (whole)	11.01	0.16	1%
Dried red chilli flakes	11.10	0.24	2%
Red chilli powder	11.02	0.21	2%

## Improved method – Soxhlet extraction

- Extractions by soaking in solvent were slow, low yielding and required large volumes of solvent
- An improved method was designed using a Soxhlet extractor.

Method:

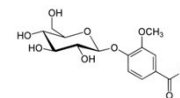
- Soxhlet extractor packed with cotton wool and 20.0g of red chilli powder.
- Ethyl acetate (60cm<sup>3</sup>) charged to collecting flask and reflux condenser fitted.
- Apparatus heated to reflux for 1h.
- Once cooled, extracted sample washed with deionized water (3 x 50cm<sup>3</sup>), dried with magnesium sulfate and evaporated to dryness.

Yield: 1.71g (8.55% by mass, viscous deep red oil)



## Magic milk? Mechanism of action for cooling spicy taste

- Milk and dairy products known to reduce the spiciness of chillies – water has no positive impact
- Two possibilities for mechanism of action:
  - Fats and proteins in milk help solubilise capsaicin away from nerve receptors
  - Capsaicin reacts with compound in milk to block p-hydroxy group (known centre for nerve binding)
- Vanillin chosen as model compound owing to similarities in aryl core
- Vanillin known to form sugar adduct under mildly acidic conditions so may react with lactose in milk!



Method:

- Vanillin (2.0g) stirred with full milk (25cm<sup>3</sup>) and heated to 35°C.
- Extracted into ethyl acetate (25cm<sup>3</sup>). Resulting dense foam washed with brine (25cm<sup>3</sup>) allowed to settle.
- Separated organic dried with magnesium sulfate and evaporated to dryness.
  - GC-MS analysis of products showed complex mixture with large amount of vanillin remaining
  - FT-IR shows additional peaks but is inconclusive.
- Washing samples of chilli extract with water showed little (<0.01g per 1g) reduction in mass.
- Washing with full milk (4% fat) showed greater reduction (0.20g per 1g), indicating likely solvation of organic components.

## Conclusions

- Efficient methods for the extraction of vanillin and capsaicin have been developed
- Analysis by GC-MS can distinguish "native" vanillin from synthetic variants
- Solubility vs reactivity studies suggest solubilisation of capsaicin is the likely mode of action for milk quenching spicy taste of chillies

## Future Work

- Investigate vanilla-flavoured products to contend "real vanilla" claims
- Further purify capsaicin from chilli extract to enable improved reactivity studies
- Synthesise vanillin analogues and conduct structure/activity studies